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FACTORS AFFECTING THE PRODUCTION OF ZYGOSPORES BY CHOANEPHORA CUCURBITARUM [1]

H. L. BARNETT AND V. G. LILLY

(WITH 5 FIGURES)

This paper is the third in a series dealing with the physiology of *Choanephora cucurbitarum* (Berk. & Rav.) Thaxter. The two previous papers (Barnett and Lilly, **1950**, **1955**) dealt primarily with the effects of nutrition and environment on growth and asexual reproduction of one isolate.

Wolf (**1917**) was the first to observe zygospores of this fungus in culture and to describe briefly their development. Its heterothallic nature was reported by Blakeslee *et al* (**1927**), who tested 33 isolates from the Cold Spring Harbor area and found that five were (+) and 28 were (−). Zygospores of *C. cucurbitarum* have also been reported in culture by Dastur (**1920**) and Sinha (**1940**). Couch (**1925**) described the development of zygospores of a species which he named *C. conjuncta*. In a recent discussion of the reproductive structures of the *Choanephoraceae*, Poitras (**1955**) concludes that *C. conjuncta* is a synonym of *C. cucurbitarum*.

The present paper reports the results of a study of the nutritional and environmental conditions which affect the production of zygospores in culture and constitutes the first report of zygospores in nature.

MATERIALS AND METHODS

Both the (+) and (−) cultures used in this study were isolated from the same pumpkin flower at Morgantown, West Virginia, in

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August 1954. Abundant conidia were obtained by growing the cultures on agar media of low sugar content under conditions of alternating light and darkness at 25° C. A constant supply of viable (+) and (−) spores was maintained by collecting them in tubes of distilled water and freezing them in a refrigerator (Barnett and Lilly, **1950**). Frozen spores remained viable under these conditions for as long as 10 to 12 months.

The basal medium used in the nutrition experiments contained the following: carbon source, 3 gms (unless otherwise stated); nitrogen source, 1 gm; KH_2PO_4, 1 gm; $MgSO_4 \cdot 7H_2O$, 0.5 gm; micro elements as sulfates (Fe, 0.2 mg; Zn, 0.2 mg; Mn, 0.1 mg); thiamine, 100 μg; distilled water, 1 liter; agar, when desired, 20 gms. Liquid media were used in 25-ml amounts in 250-ml Erlenmeyer flasks. All media were adjusted to pH 6.0, except in the pH experiments, and autoclaved at 15 pounds pressure for 15 minutes. Spore suspensions in distilled water were used as inoculum.

EXPERIMENTAL RESULTS

Identification of (+) and (−) cultures. Zygospores were observed in one of the isolation plates derived from conidia on a wilted pumpkin flower. From this plate five single-conidium cultures were obtained from each of 17 conidial heads. By pairing certain cultures it was determined that all isolates from the same conidial head were of the same sex. Seven conidial heads belonged to one sexual group and 10 belonged to the opposite group.

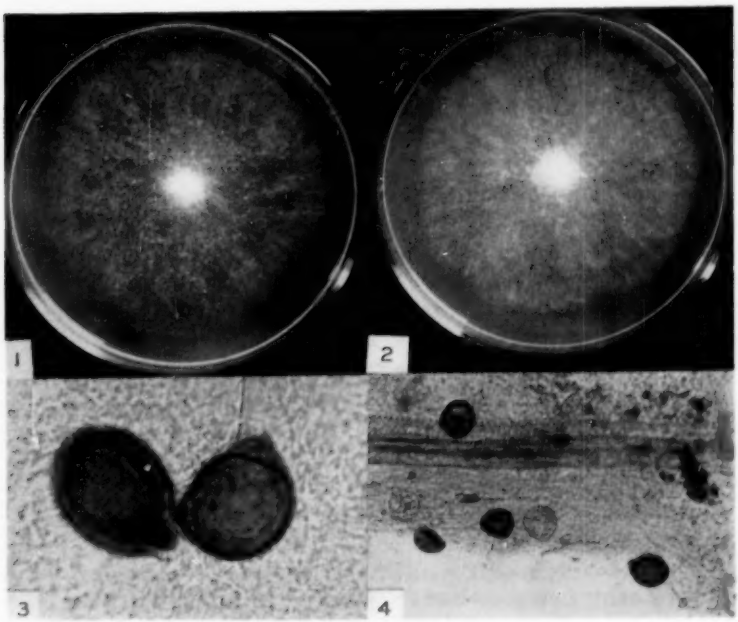
All single-spore cultures appeared alike when grown on malt extract-yeast extract agar, but on glucose-asparagine agar the mycelium of one sexual group produced small tufts of hyphae, giving a slightly rough appearance (FIG. 1). The mycelium of the other group was much smoother in appearance (FIG. 2).

The correct designations of (+) and (−) to the sexual groups could be made only by pairing each with known (+) and (−) cultures of a related fungus. In this case, *Phycomyces blakesleeanus* was used, according to the technique of Blakeslee (**1915**). Stimulated production of enlarged branches, believed to be abortive progametangia, on a line between the two species was considered as a sexual reaction. Thus, it was determined that the "rough-mycelium" cultures were (−) and the "smooth" cultures were (+).

Development of zygospores. When a suitable agar medium was inoculated at two points with (+) and (−) spores or mycelium, progametangia began to form in a zone as soon as the hyphae made contact.

The first zygospores matured within 48 hours. The zone of zygospores continually widened as the (+) and (−) mycelia intermingled. Abundant conidia were formed on each mycelium before the two met, but their production ceased when zygospores began to form.

Agar plates flooded with a suspension of mixed (+) and (−) spores produced gametangia within 24 hours and few sporangia or conidia



FIGS. 1–4. *Choanephora cucurbitarum*. 1. Culture of (−) sex two days old on glucose-asparagine agar. Note the small tufts of hyphae giving it a rough appearance. 2. Culture of (+) sex two days old on glucose-asparagine agar. Note the smooth appearance of the mycelium. 3. Zygospores produced in culture. The one on the left shows the surface ridges and the one on the right shows the large central oil droplet. 4. Zygospores produced in nature in well-rotted pumpkin flower.

were formed. Thus, sexual reproduction occurred at the expense of asexual reproduction under these conditions. On malt extract-yeast extract agar the number of zygospores, as estimated by counts of five representative fields under the stereoscopic microscope, ranged from 200,000 to 400,000 per Petri dish culture. They were submerged in the agar as well as on the surface.

The presence of ridges or striations on the zygospore wall, reported recently by Poitras (**1955**), was confirmed (FIG. 3). These markings were prominent on zygospores produced on agar media near optimum temperatures.

Effects of temperature and light. Since both light and temperature are known to effect the production of asexual spores of *C. cucurbitarum* (Barnett and Lilly, **1950**, **1955**), it was desirable to determine their influence on the formation of zygospores. Plates of malt extract-yeast extract and glucose-asparagine media were inoculated by flooding with a suspension of mixed (+) and (−) spores and four plates of each were incubated in continuous light and continuous darkness at 10, 15, 20, 25, 30, and 35° C.

Gametangia were formed quickly under all conditions except at 10° and 15° C. The approximate time required for the production of mature zygospores was as follows: 35° C, 4 days; 30° C, 2 days; 25° C, 3 days; 20° C, 6 days; 15° C in darkness, 10 to 12 days. In continuous light at 15° C, only an occasional zygospore was formed. No zygospores were present at 10° C. Exposure to light at other temperatures had no apparent effect. Fewer zygospores were formed near the limits of the temperature range than near the optimum. In supplementary experiments it was found that zygospores begin to form at 37° C but few reach maturity at this temperature. In liquid media in flasks, zygospores failed to form at temperatures of 35° and at 15° C.

Effect of increased carbon dioxide. This factor was tested by placing inoculated agar plates in sealed desiccators. The desired percentage of carbon dioxide was approximated by first reducing the air pressure in the desiccator by means of a pump, adding the desired amount of pure carbon dioxide from a tank of compressed gas and then allowing air to enter the desiccator to bring the pressure back to normal.

After 4 days abundant mature zygospores were present in cultures growing in air and in an atmosphere containing approximately 4 percent carbon dioxide. In 10 percent carbon dioxide the number of zygospores was greatly reduced and the maturation delayed, but growth of mycelium was reduced only slightly. In an atmosphere containing approximately 20 percent carbon dioxide growth was greatly reduced and there was no sign of sexual reproduction. The effect of increased carbon dioxide was not modified by the addition of pure oxygen in amounts equalling the added carbon dioxide.

Effect of carbon source. Preliminary experiments showed that zygospores formed when the mycelium was completely submerged in liquid media either in standing or in shake cultures, but they were produced

more slowly than on agar. An increase in the concentration of sugar caused a delay in the formation of gametangia and in maturation of zygospores. Liquid synthetic media used in these experiments contained 5 gms sugar and 1 gm asparagine per liter. Ten sugars were compared.

Zygospores were produced in all media, but the time and abundance varied with the sugar. They were formed quickly in media containing sucrose, maltose (C P), lactose and raffinose, which are poor for vegetative growth. Zygospores formed more slowly, although they were

TABLE I

DRY WEIGHT OF MYCELIUM, pH AND DEVELOPMENT OF ZYGOSPORES IN LIQUID MEDIA CONTAINING DIFFERENT NITROGEN SOURCES AFTER 4 DAYS, AND NUMBER OF DAYS REQUIRED FOR FIRST ZYGOSPORES TO REACH MATURITY. INITIAL pH OF MEDIA WAS 6.0

Nitrogen source	Mg dry mycelium	pH after 4 days	Zygospores 4 days	Days required for matured zygospores
Ammonium nitrate	27	3.1	none	—*
Ammonium sulfate	23	3.3	none	—
Ammonium tartrate	21	4.1	none	—
L-asparagine	24	7.1	nearly mature	5
DL-alanine	28	6.6	immature	8
L-arginine	31	6.6	none	7
L-glutamic acid	32	7.6	some mature	3
Glycine	trace	6.6	none	12
L-leucine	3	6.6	none	8
DL-phenylalanine	2	6.7	mostly mature	3
L-proline	40	6.3	immature	7
Urea	20	8.3	none	—

* No zygospores present at 14 days.

more numerous in media containing D-glucose, D-fructose, D-galactose and cellobiose. Both L-sorbose and D-xylose were poor for growth and zygospore production.

Effect of nitrogen source. Ten nitrogen sources were tested at the rate of one gm per liter in media containing 5 gms glucose in stationary liquid cultures. The response to different nitrogen sources was more varied than the response to different sugars. The results are summarized in TABLE I. No zygospores were present within 14 days in media containing ammonium compounds or urea, although a few abnormal, immature zygospores formed later in urea and ammonium tartrate media. Zygospore formation was most rapid in glutamic acid, asparagine and phenylalanine media, although little mycelium was present in the phenylalanine medium. It is of interest that mature

zygospores were produced on agar media containing each of the ten nitrogen sources.

The effect of acidity. The possible importance of favorable pH range of around 6.5 to 8.0 is suggested by the results in TABLE I. However, the absence of zygospores in media containing ammonium compounds and urea could be due to the nitrogen sources. In order to determine which factor was unfavorable, it was necessary to maintain a constant favorable pH of the media throughout the growth period of the fungus, and also to grow the fungus on one medium at different pH levels.

A slow continuous flow of liquid medium over the mycelium was selected as one of the most accurate ways of maintaining a constant pH. A simple but specialized culture tube for this purpose was designed and prepared from a 20 mm test tube (FIG. 5). To this sterile tube approximately 3 ml of melted agar medium of desired composition was added and allowed to solidify in a horizontal position. Following inoculation with a suspension of mixed (+) and (−) spores, an incubation period of 12 to 15 hours permitted the spores to germinate and the hyphae to become anchored in the substrate. Liquid medium from a 2-liter reservoir flask was allowed to drip from a glass capillary tube into the vertical arm of the culture tube. The rate of flow was maintained at about 12 to 15 drops per minute by adjusting the height of the reservoir flask. The pH of the liquid medium was changed but little by passage over the growing mycelium.

Mycelium continuously supplied with fresh medium at pH 6.0 completely filled the culture tube within three days, but failed to produce zygospores. This result is similar to those found for some other fungi which failed to sporulate in the presence of a plentiful supply of nutrients, but sporulated readily when starved by removal of the medium or exhaustion of the carbon source (Barnett and Lilly, **1947**; Timnick, *et al*, **1952**).

The procedure was then modified to allow a period of 24 hours for the establishment of the mycelium, which was then starved by replacing the medium with distilled water. Zygospores then began to form within 24 hours and were mature within 48 hours. Using this method, cultures were grown in media (malt extract 1 gm, yeast extract 0.5 gm) at pH levels of 3.5 to 8.5. These media were replaced by a phosphate buffer solution (1 gm KH_2PO_4 per liter distilled water) at the same pH as the culture medium. Zygospores were produced quickly within a pH range of 4.5 to 8.5, developing more rapidly in the less acid media. A few immature zygospores were present at pH 4.0 after two days, but none developed at pH 3.5.

Synthetic media were then used, with glucose and each of four nitrogen sources, ammonium sulfate, asparagine, glutamic acid and urea. The pH was 6.0. Under these conditions zygospores were produced in ammonium sulfate and urea media as readily as in glutamic acid and asparagine media. Thus, the failure to form zygospores in still liquid

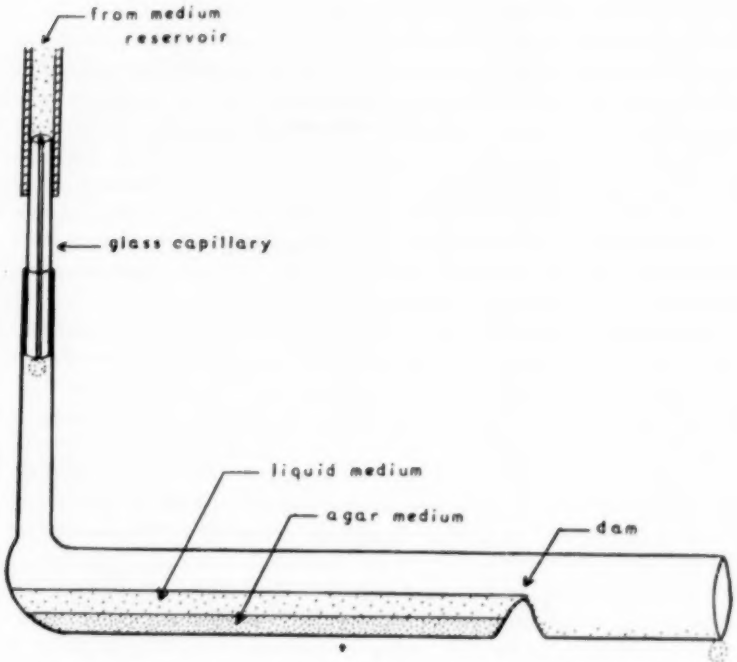


FIG. 5. Special culture tube used for continuous flow of liquid medium over growing mycelium. The medium is siphoned through glass and rubber tubes, passes through the glass capillary and drips to the culture tube. Uniform rate of flow can be maintained by adjusting the height of the reservoir flask.

culture containing certain nitrogen sources was due to an unfavorable pH.

Association of beta-carotene. During routine culturing of this fungus in liquid media, it was noted that the mycelium in mixed (+) and (−) cultures became bright yellow, while the mycelium of each sex when grown separately was only slightly yellow. The yellow pigments were extracted and determined to consist chiefly of *beta*-carotene.

Quantitatively, on the basis of dry weight of mycelium, the mixed cultures produced 15 to 20 times as much *beta*-carotene as did either grown separately (Barnett *et al*, **1956**). Both the (+) and (−) cultures showed increased carotene production when grown on opposite sides of a cellophane membrane. It was concluded that the production of carotene was stimulated in either culture by hormone-like substances originating from the mycelium of the opposite sex.

In still liquid culture maximum carotene production was reached just prior to or during zygospore formation. Microscopic study revealed a concentration of reddish-orange granules of carotene in the suspensors. Wolf (**1917**) noted the presence of yellowish oil globules in immature zygospores, but it is not known how much *beta*-carotene enters with the oil. These observations suggest a possible relationship between *beta*-carotene and sexual reproduction. This subject is discussed briefly by Goodwin (**1952**). The addition of diphenylamine, a known carotene inhibitor, to the culture medium resulted in a delay of both carotene production and zygospore formation.

Production of zygospores in nature. Zygospores of *C. cucurbitarum* have been reported only from culture. The occasional isolation of both (+) and (−) mycelia from the same infected flower suggests that zygospores may be formed frequently in nature. In late summer of 1955 this fungus was abundant on cucurbit flowers and young fruits in the vicinity of Morgantown. From one garden fading flowers of pumpkin and gourd bearing conidia were collected in separate bags. Isolations were made from a few conidial heads from each of 42 flowers and the cultures from each flower were grown on the same agar plate. Zygospores appeared in 20 of these plates. This shows that both sexes of the fungus were present on about half of the infected flowers.

An examination was then made for the presence of zygospores of this fungus in naturally infected pumpkin flowers. Moist decaying flowers bearing conidia were collected and examined microscopically for zygospores. In searching through some 30 to 40 flowers, mature zygospores of *C. cucurbitarum* were found in three (FIG. 4). The zygospores were easily recognized by the large oil drop and the faint striations on the wall. The formation of zygospores in host tissue was confirmed by artificial inoculations of flowers and young fruits in the laboratory.

DISCUSSION

The present study has revealed a number of interesting facts regarding the physiology of zygospore formation by *C. cucurbitarum*.

The production of conidia, the asexual stage commonly found in nature and in culture, is influenced by a number of environmental factors, including temperature, light, humidity and amount of carbon dioxide (Barnett and Lilly, **1950**, **1955**). In contrast, the formation of zygospores, when both (+) and (−) mycelia are present, is affected by relatively few factors of the environment.

Some of the conditions which permit zygospore formation but which prevent or greatly reduce the production of asexual spores are as follows: (1) continuous darkness; (2) continuous light, except near minimum temperature; (3) extremes in temperature; (4) increased carbon dioxide in the atmosphere to 4 percent; (5) culturing in liquid media. On agar plates inoculated by flooding with a mixture of (+) and (−) spores, zygospores formed in great numbers at the expense of the asexual stages. It is unusual among fungi having both types of reproduction that sexual reproduction should occur under so many conditions unfavorable to asexual reproduction. The ready formation of zygospores submerged in liquid media seems to be unusual among the Mucorales. It may represent an adaptation to zygospore formation in nature in the wet, well-rotted host tissue.

The production of zygospores is favored by a medium low in sugar content. Growth of the mycelium continues as long as it receives an adequate supply of food, but zygospores begin to form within 24 hours after the mycelium is starved.

No correlation was found between utilization of nitrogen sources for growth and for zygospore production. A wide variety of nitrogen sources were favorable for zygospore production, provided that the pH was maintained within a favorable range. The use of specially designed culture tubes, through which the medium flowed continuously, served as a simple way to maintain constant pH, and to distinguish between the effects of nitrogen source and acidity. The favorable pH range of 4.5 to 8.5 is of interest because it is unusually wide.

A possible relation between *beta*-carotene and sexual reproduction is indicated, but the evidence is not conclusive. It is based on (1) the increased production of *beta*-carotene believed to be stimulated by hormone-like substances originating from the mycelium of the opposite sex; (2) the concentration of carotene in the suspensors; and (3) the effects of diphenylamine in delaying zygospore formation as well as inhibiting carotene production. It seems that the formation or maturation of zygospores is in some way dependent upon the production of *beta*-carotene, or that both physiological processes are affected by the same general nutritional and environmental conditions.

The characteristic longitudinal striations on the wall was an aid in identifying zygospores found in nature. However, zygospores developed in culture at temperatures near the maximum and minimum appear to be entirely smooth. It is possible that they never completely mature at these temperatures.

The presence of zygospores of *C. cucurbitarum* in naturally infected host tissue is believed to be reported here for the first time. The importance of the zygospore stage in the life of the fungus is still unknown, and the conditions necessary for the germination of zygospores have not been determined.

SUMMARY

The mycelia of the (+) and (−) sexes of *C. cucurbitarum* isolated from the same source were found to differ slightly in appearance on certain media.

In the presence of mixed (+) and (−) mycelia zygospores develop abundantly within a few days on a wide variety of agar and liquid media. The range of favorable temperature was approximately 15–37° C. Light was not required and had no apparent effect, except at 15° C, where it was inhibitory. Zygospores formed in an atmosphere containing as much as ten percent carbon dioxide.

Zygospore production coincided with the exhaustion of the available carbon supply in the medium and was induced by subjecting well nourished mycelium to starvation conditions. Some sugars which were poor for mycelial growth favored quick zygospore production. All nitrogen sources tested favored their formation, provided the medium did not become too acid. The favorable pH range was approximately 4.5 to 8.5.

A simple culture tube designed for the slow continuous flow of liquid medium over growing mycelium is described. This method was used to maintain constant pH of the medium.

In mixed cultures of (+) and (−) mycelia, particularly in liquid media, there is produced a greatly increased amount of *beta*-carotene, apparently stimulated by hormone-like substances secreted by the mycelium of the opposite sex. The *beta*-carotene becomes concentrated in the suspensors and is suspected of having some function in sexual reproduction of this fungus.

Mature typical zygospores were found in moist, well-decayed pumpkin flowers collected from a garden where both sexes had been found previously.

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EFFECT OF IRON, ZINC, MANGANESE AND CALCIUM ON THE GROWTH OF VARIOUS STRAINS OF STREPTOMYCES

ALLEN H. HEIM AND HUBERT LECHEVALIER

(WITH 1 FIGURE)

Although members of the genus *Streptomyces* have become extremely important as producers of antibiotics comparatively little is known about their microelement requirements. Most studies of this nature were directed principally toward the elucidation of the microelement requirement of a specific *Streptomyces* strain for the production of a specific antibiotic, and most of the literature dealing with this subject has been reviewed in previous papers (**1**, **4**). Recently, Spicher (**3**) reported that the growth-promoting action of soil extracts on strains of *Streptomyces* was the result of the trace element content of the extract. He found Fe, Mn, Zn, Cu, and Mo effective and observed that the elements did not act singly but in combinations. The combinations Fe-Zn, Fe-Mn-Zn, and Fe-Mn-Zn-Mo were the most effective.

The aim of the present investigation was to compare the effect of four elements (Fe, Zn, Mn, and Ca) on the growth of eight different strains of *Streptomyces* and to determine the extent of their interactions.

MATERIALS AND METHODS

The cultures studied were: *Streptomyces fradiae* No. *3535*, *S. fradiae* No. *3556A*, *S. griseus* No. *3475*, *S. aureofaciens* No. *3550A*, *S. flaveolus* No. *3319*, *S. lavendulae* No. *3555*, *S. rimosus* No. *3558* and *S. coelicolor* No. *3030*. All culture numbers refer to the collection of the Institute of Microbiology of Rutgers University.

The "distilled" water used was single distilled water of 250,000 to 300,000 ohms resistance. "Distilled-deionized" water was obtained by passing this water through a column composed of a mixture of synthetic cation and anion exchange resins (Rohm and Haas, Amberlite MB-1, analytical grade). Water of 3 million ohms resistance was consistently obtained by this method. The conductivity of the water was measured with a type RC (Industrial Instrument) conductivity bridge.

All the glassware used was of "Pyrex" brand and was washed in distilled water, soaked for at least 24 hours in 3N HCl, rinsed twice with distilled water and three times with distilled-deionized water, and subsequently drained dry.

Routine pH determinations were made with Hydrion short range pH test paper and were checked occasionally with a Beckman model G pH meter.

Growth was measured by weighing the dry mycelium. The mycelium was collected by vacuum filtration of the cultures through No. 2 Whatman filter paper disks (5.5 cm) which had previously been dried for 24 hours at 100° and tared. After filtration, the paper disks were dried again at 100° for 24 hours and weighed. This procedure was checked in order to determine that constant weight had been obtained and that no loss of weight of the tared paper disks occurred during the washing with the medium and with water.

The medium devised for this study supported adequate growth of all the selected strains of *Streptomyces*, and was of the following composition:

Dextrose (Corn Products Anhydrous "Cerelose")	10 g
Glycine (Nutritional Biochemicals Corp.)	10 g
L-glutamic acid (Merck)	5 g
Casein hydrolysate, vitamin free (Nutritional Biochemicals Corp.), 10% solution	10 ml
K_2HPO_4 ("Baker analyzed" reagent)	0.5 g
$MgSO_4 \cdot 7H_2O$ ("Baker analyzed" reagent)	0.2 g
$CuCl_2 \cdot 2H_2O$ ("Baker analyzed" reagent)	3 mg as Cu
$FeCl_3 \cdot 6H_2O$ ("Baker analyzed" reagent)	3 mg as Fe
$CaCl_2 \cdot 2H_2O$ ("Baker analyzed" reagent)	3 mg as Ca
$MnCl_2 \cdot 4H_2O$ ("Baker analyzed" reagent)	3 mg as Mn
$ZnCl_2$ (Merck Reagent)	3 mg as Zn
Distilled-deionized water	to 1 liter

The method of treating the medium to remove as much of the contaminating trace metals as economically possible was a modification of the alumina method advocated by Donald et al. (2). The method finally adopted is given here for one liter of medium. The necessary amounts of dextrose, glycine, L-glutamic acid, casein hydrolysate, K_2HPO_4, and $MgSO_4$ were each dissolved separately in distilled-deionized water to a volume of 100 ml. The pH of the glycine, glutamic acid, and casein hydrolysate solutions was adjusted with NaOH to 7.8–8.0. Two percent alumina (activated chromatographic, powdered catalyst grade, Harshaw Scientific) was added to each solution, the flasks were swirled, and autoclaved (18 lb for 25 minutes). After autoclaving, the flasks were swirled again and allowed to stand overnight. The alumina was removed the next day by filtration through

a "fine" sintered glass filter. The various components of the medium, with the exception of the dextrose, were combined and made up to volume (900 ml) with distilled-deionized water. The medium was apportioned into 250 cc Erlenmeyer flasks to the level of 90 ml per flask. Trace metals were added as needed from stock solutions containing 3 mg of the metal per ml. The flasks were covered with inverted 50 cc beakers. The medium was then autoclaved (8 minutes at 18 lbs). The dextrose solution was autoclaved separately in the same manner. After sterilization the dextrose was added. The final volume was 100 ml per flask and the final pH 7.6 to 7.8. The medium was prepared in batches of five liters as was needed for each experiment.

The previously described medium, without addition of Fe, Ca, Mn, and Zn, will be referred to as the "deficient" medium. This medium with the addition of all four metals will be referred to as the "complete" medium.

Response of the streptomycetes in the form of low mycelial weights in the deficient medium indicated that the alumina method of deionization was relatively efficient. A spectrographic analysis further substantiated this observation. It was found that the medium was qualitatively equal to spectrographically standardized chemicals.

Inocula were prepared by transferring the spores from a slant to two flasks of the deficient medium. These flasks were inocubated on a rotary shaking machine at 28° for 7 days. The two cultures were then combined and used directly as the inoculum in the case of *S. fradiae*, *S. rimosus*, and *S. flaveolus*. In the case of *S. aureofaciens*, *S. griseus*, *S. lavendulae*, and *S. coelicolor*, the cells of the inocula were washed 3 to 5 times in distilled-deionized water before being used as the inoculum. The amount of inoculum was essentially the same for every flask of a given experiment but varied from 0.5 mg to 1.8 mg per flask from one experiment to the other. Each experiment consisted of seeding with one strain of *Streptomyces* 48 flasks (16 media in triplicate) and incubating the flasks at 28° on rotary shaking machines (235 RPM.; eccentricity 0.5 inch). Collections of the mycelium were made as close as possible to the stage of maximum growth, a point determined by pilot experiments run on the complete medium.

RESULTS AND DISCUSSION

The design of the experiments was factorial. The 16 media included the deficient medium, four media containing a single metal, six media containing a combination of two metals, four media containing the

metals in combinations of three, and the complete medium. The effect of the various metals and combination of metals on the growth of the streptomycetes was evaluated on analysis of the factorial experiments (5).

The arithmetic means of the three replicate yields of dry mycelium obtained on the various media for each organism are listed in TABLE I. One experiment was run with each of the organisms except for *S. fradiae 3535* and *S. aureofaciens 3550A*, for which two independent sets of data are available. Thus, TABLE I gives the results of ten separate factorial experiments.

TABLE I

WEIGHTS OF DRY MYCELIUM, IN MG PER 100 ML OF MEDIUM, PRODUCED BY EIGHT STRAINS OF STREPTOMYCES IN 16 MEDIA. EACH FIGURE IS THE AVERAGE OF THREE FLASKS

Addition to deficient medium \ Strains	*3535*		*3556A*	*3319*	*3030*	*3555*	*3550A*		*3558*	*3475*
	1*	2*					1*	2*		
O	18	19	25	21	25	29	49	63	68	80
Fe	88	202	76	93	123	70	155	162	181	98
Zn	12	6	21	26	19	30	43	51	98	115
Mn	13	16	16	18	26	30	41	47	60	76
Ca	12	18	21	24	30	30	45	57	72	81
Fe, Zn	338	184	297	167	161	231	209	246	176	264
Fe, Mn	68	93	69	72	137	57	76	145	116	95
Fe, Ca	57	118	103	134	133	65	89	113	167	103
Zn, Mn	11	5	10	20	27	28	44	53	90	134
Zn, Ca	10	11	14	29	33	38	51	55	88	123
Mn, Ca	13	19	19	24	38	29	41	46	68	83
Fe, Zn, Mn	249	310	291	166	176	251	305	233	247	259
Fe, Zn, Ca	127	395	367	212	256	267	265	235	261	323
Fe, Mn, Ca	51	121	99	127	151	64	79	121	151	72
Zn, Mn, Ca	14	17	21	27	42	26	42	45	101	145
Fe, Zn, Mn, Ca	266	326	379	231	295	256	268	226	282	340

* 1 and 2 refer to duplicate experiments.
3535 and *3556A* = *S. fradiae*; *3319* = *S. flaveolus*; *3030* = *S. coelicolor*; *3555* = *S. lavendulae*; *3550A* = *S. aureofaciens*; *3558* = *S. rimosus*; *3475* = *S. griseus*.

One will note that the amount of cellular material obtained in the deficient medium varies from one *Streptomyces* to another. Also, the ratios obtained by dividing the weight obtained on the complete medium by the weight obtained on the deficient medium vary from one culture to the other. Three possible explanations for this variation come to mind: (1) the glassware and the medium were not consistently free of contaminating metals, (2) the various strains of *Streptomyces* vary in their quantitative microelement requirement, or (3) the various strains vary in their qualitative microelement requirement. The available data permit one to discard at least the first hypothesis since growth in the

deficient media of the duplicate experiments run a few weeks apart with *S. fradiae 3535* and *S. aureofaciens 3550A* checked fairly well. *S. griseus 3475* was used in most of the preliminary experiments involved in the elaboration of the method for washing glassware and removing trace elements from the medium; we thus have data beyond those reported here which show that this organism always gave, comparatively speaking, high mycelial yield in the deficient medium.

From the data reported in TABLE I, one will note that iron is the only metal which when added *singly* consistently produced a marked increase in mycelial weight over that obtained in the deficient medium.

TABLE II

ESTIMATES OF THE MEAN EFFECT [1] OF FE, ZN, MN, CA AND THE INTERACTION FE-ZN IN THE TEN EXPERIMENTS LISTED IN TABLE I

Strains \ Source of variation	Fe	Zn	Mn	Ca	Fe-Zn
S. fradiae Expt. No. 1	143	88	—	—	91
3535 Expt. No. 2	205	81	—	24	89
S. fradiae 3556A	192	122	—	27	125
S. flaveolus 3319	126	46	—	28	42
S. coelicolor 3030	149	43	—	35	43
S. lavendulae 3555	127	94	—	—	93
S. aureofaciens Expt. No. 1	136	81	—	—	80
3550A Expt. No. 2	133	49	—	−13	51
S. rimosus 3558	117	57	—	19	23
S. griseus 3475	93	125	—	23	77

[1] The mean effect of a factor is the mean of the difference observed between media where this factor is present and media where this factor is absent.
All figures listed in the table are significant at the 0.1% level.

It is difficult to visualize from the results, as reported in TABLE I, the effect of the various metals and metal interactions on the growth of the streptomycetes. This can be more easily accomplished by referring to TABLE II where the estimates of the mean effects of the metals are presented. The very striking beneficial effect of the iron-zinc interaction warranted its inclusion in the table.

The mean effect of a metal is the mean of the difference between the growth in all the media where this metal is present and all the media where this metal is absent. It is the advantage of the factorial design of these experiments that, although only 48 flasks were used per experiment, there was for each one of the four metals studied 24 flasks containing the metal and 24 flasks not containing the metal. By a simple series of additions and subtractions an estimate of the effect of each metal or each interaction between metals can be obtained (**5**).

The over-all effect of manganese was negligible, but at times the interaction manganese-zinc was most favorable. One should remember that all these data were obtained on a magnesium-containing medium on which the need for manganese might be nil, since manganese and magnesium can be substituted for one another in many enzymatic systems. It was not found possible, however, to replace magnesium by manganese in the medium used.

The over-all effect of calcium in this medium, which probably is not truly calcium-deficient, since traces were detected spectrographically, was sometimes favorable and sometimes unfavorable. Such marked discrepancies were observed between the replicate experiments run with *S. fradiae 3535*. It is felt that this lack of consistency in the effect of calcium and its various interactions may be attributed to an inadequate experimental design. There is indeed no *a priori* reason why the growth and lysis of streptomycetes should progress at the same rate on the sixteen media studied. By collecting the growth after only one period of incubation, one gets only the picture at that time. As such, the risk exists that in certain media growth measurements were made before maximum growth was obtained, whereas in other media, measurements were made after the start of lysis. From the pH measurements it can be postulated that on the whole the various cultures were collected before they had attained their full development. For this reason we believe that the observed mean effects should be interpreted with caution and that this experimental design should not be expected to show any more than the most marked effects.

In order to determine whether the apparent effect of calcium may vary with the time of incubation, additional experiments were run with *S. fradiae 3535* grown on nine media: the deficient medium and all the iron-containing media. Fifteen flasks of each medium were inoculated. After 5, 6, 7, 8, and 10 days of incubation, the flasks were removed by groups of three and the dry mycelial weights were determined. Growth curves obtained in one of these experiments are shown in FIG. 1. As expected, lysis occurred sooner in certain media than in others. Lysis occurred early and rapidly in the iron-zinc medium, and late and slowly in the iron-zinc-calcium medium. The estimate of the over-all mean effect of calcium was found to be + 17.1 at 6 days, the effect having no statistical significance, and + 160.2 at 10 days, this result being significant at the 0.1% of significance. This demonstrates clearly the inadequacy of our previous experiments for the elucidation of the over-all effect of calcium on the growth of the strains of *Streptomyces*.

The striking effect of iron in all cases causes one to suspect the

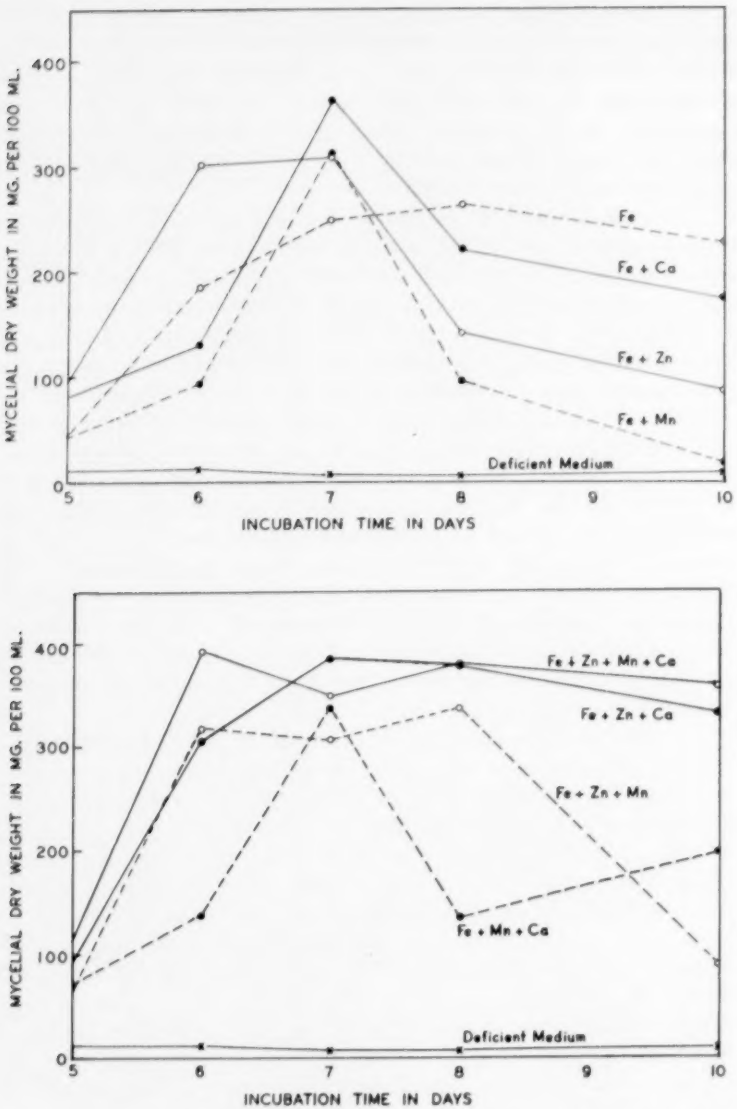

FIG. 1. Growth of *S. fradiae 3535* in the deficient medium and in 8 media containing metals added as indicated.

presence of iron-containing compounds, possibly cytochromes. An examination with a hand spectroscope reveals the presence of an absorption band at 550–560 mμ in suspensions of these streptomycetes when reduced with sodium hydrosulfite. This is presumptive evidence for the presence of iron-porphyrins. However, extraction has not been effected. At present the exact nature of the compound or compounds is undetermined.

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SUMMARY

1. A study was made of the effect of four metals, iron, zinc, manganese, and calcium, on the growth of eight different strains of *Streptomyces* grown in the same medium in shake flasks. The design of the experiments was factorial in order to permit the analysis of the effect of possible interactions.

2. Iron was the only element that permitted a substantial increase in mycelial weight when added singly.

3. Zinc and the interaction iron-zinc had a marked favorable effect on growth.

4. Further experimentation will be needed to elucidate the effect of manganese and calcium.

5. The rate of growth and the rate of lysis of *S. fradiae 3535* was found to vary with the metal composition of the medium; calcium delayed lysis.

6. The final level of growth attained by different strains, under comparable environmental conditions, varied greatly, indicating significant strain differences in quantitative and/or qualitative mineral requirements.

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SOME VIEWPOINTS ON THE PHYLOGENY OF RUST FUNGI. II. GYMNOSPORANGIUM [1]

E. E. Leppik

(WITH 4 FIGURES)

The genus *Gymnosporangium* is a most noteworthy group among the "true" aecioid rust fungi.[2] Ordinarily these rusts pass their aecial stage on conifers and their teliophase on angiosperms, but *Gymnosporangium* produces teliospores on Cupressaceae and aecia on the *Rosales*.

The development of cornute and roestelioid aecia is a further new tendency. These aecia have a mechanism which regulates the expulsion of spores in accordance with changing weather conditions. No other plant rust is known to have such an advanced mechanism. Of further interest are the jelly-like matrices in which the teliospores are imbedded.

An extensive taxonomic and biologic treatise of the genus *Gymnosporangium* was written by Kern (**13**, **14**). Numerous further research records published by Dodge (**6**, **7**) and other authors are summarized by Buller (**4**, pp. 389–414). Despite this intensive study, both the origin of the genus *Gymnosporangium* and its phylogeny remained relatively obscure.

[1] This study has been carried out at Augustana College, Sioux Falls, South Dakota, and was supported in part by research grants of the Mycological Society of America during the years 1953, 1954 and 1956 (see Acknowledgments). The first report of this study was published in Mycologia **45**: 46–74, 1953. During the printing of this report the author received some papers on a closely related subject from D. B. O. Savile of Ottawa: "A phylogeny of the Basidiomycetes" in Canad. Journ. Bot. **33**: 60–104, 1955, and others. In these well-reasoned articles the same concept of the evolutionary correlation between rust fungi and their host plants is approached from a somewhat different angle. The fact that these independent studies lead to the same results, in spite of different materials and methods, is highly encouraging for the validity of this novel approach to evolutionary studies.

[2] By "true" aecioid rusts is meant in this article Melampsoraceae and Pucciniaceae of Dietel (**5**), pr. part. There are, however, among Pucciniaceae primitive stomatosporous forms on tropical ferns without ordinary aecial stage (for example, *Desmella*). In another paper (**17**) these primitive forms have been joined provisionally into a special group named Stomatosporae. Further exceptions are *Cystopsora*, *Zaghouania*, and *Skierka*, with different aecia and aeciospores than in Pucciniaceae. The evidence is therefore strong that these genera are not closely related to the above-mentioned "true" aecidioid rusts and should be excluded from the family Pucciniaceae.

However, a new approach is possible by the application of the hologenetic method (**22**), which helps to establish the relative phylogenetic age of rust fungi and their host plants. Although there is only meager evidence of their geological age (Meschinelli, 1898; Dietel **5**, p. 33), the evolution of aecioid rusts is nevertheless fixed in the history of conifers—trees which provide the best known fossils.

Knowledge of biological specialization permits the establishment of hologenetic ladders (**22**, p. 58) which better reflect the evolutionary trends. In the case of unilateral pleophagia, these ladders have helped to determine the relative phylogenetic age of differnt groups of host plants (**18**).

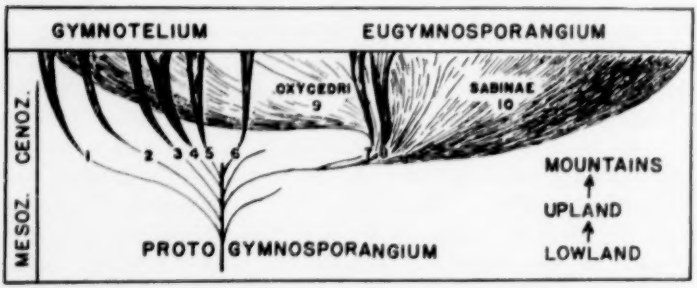


FIG. 1. Phylogenetic roots of the genus *Gymnosporangium*. Sections: 1. *Nootkatense*. 2. *Libocedri*. 3. *Ellisii*. 4. *Biseptatae*. 5. *Miyabei*. 6. *Cupressi*. 7. *Speciosae*. 8. *Clavipes*. 9. *Multiporae* (*Oxycedri*). 10. *Eutelium* (*Sabinae*).

AECIAL TYPES OF GYMNOSPORANGIUM

Because of their peculiar beak-like shape, the aecia on pear leaves have been described as a special type and named *roestelia*. Later, species of *Gymnosporangium* have been discovered with *cornute, cylindrate, cupulate, tubulate, protuberate, clathrate, cancellate, basculate*, and *balanate* aecia. This is an unexpected abundance of new types among "true" aecioid rusts.

In most cases the anatomical structure of peridial cells, particularly those of the apex, indicates their common origin from rhomboid cells of ordinary cupulate aecia. This fact indicates the close relationship of *Gymnosporangium* with "true" aecioid rusts. The protuberate type (FIG. 2: *3, 4*), however, is an exception. Its peridial cells are hyphae-like. This type is presumably a new formation from mycelium.

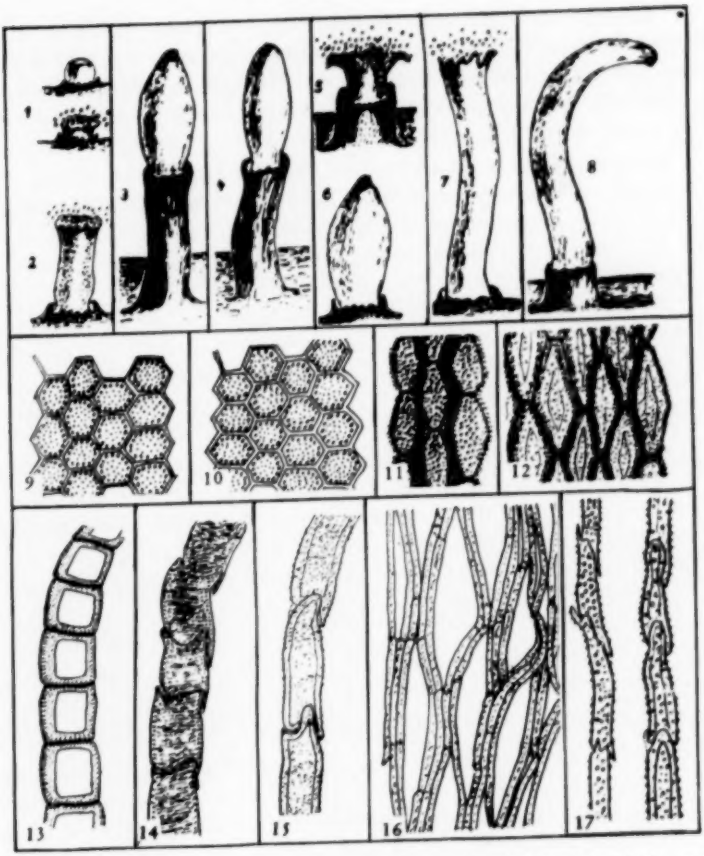


FIG. 2. Main aecial types and articulations of peridial cells of the genus *Gymnosporangium*. 1. Cupulate-type of *G. libocedri*. 2. Cylindrate-type of *G. ellisii*. 3. Protuberate-type of *G. transformans*. 4. Strobilate-type of *G. bisseptatum*. 5. Basculate-type of *G. juniperinum*. 6. Balanate-type of *G. sabinae*. 7. Tubulate-type of *G. haraeanum*. 8. Cornute type of *G. harknessianum*. 9. Hexagonal cells of a cupulate peridium of *G. libocedri*. 10. The same of a cylindrate type of *G. ellisii*. 11. Sculpture and articulation of the peridial cells of *G. haraeanum*. 12. The same of *G. confusum*. 13. Flat joint of peridial cells of *Puccinia graminis*. 14. Scarf joint (Basculate type) of *G. juniperinum*. 15. Double-notch joint (balanum type) of *G. nelsoni*. 16. Hyphoid joint (protuberate type) of *G. transformans*. 17. Double-notch and overlapping joint (balanate-type) of *G. sabinae*.

The cell wall sculpture of peridial cells is described by Kern (**13**, p. 426) as follows: rugose, verrucose, verruculose, spinulose and smooth. Although this distinctive sculpture provides perfect characteristics for the identification of species, it cannot be used with the same effect for the classification of sections and subgenera.

Apex is the top of an aecium and tip of a cornute roestelium. The *aecial apex* is formed from the same kind of peridial cells without special articulation. It ruptures under the pressure of maturated spores. From the aecial apex develops gradually a special, unbreakable *apicular cap* of roestelioid peridium, like that of the balanoid type.

Articulation of peridial cells is characteristic of the different aecial types. The peridial cells of the aecioid types (cupulate and cylindrate aecia) are abutted hexagonally and without a special articulation (FIG. *2*: *9, 10, 13*). In the cornuoid types *overlapping* and *scarf* articulations are common.

Most advanced are the articulations of the roestelioid types. In the basculate aecium, peridial cells form long rods and ribbons. Due to their flexibility these ribbons close or open the mouth of the peridium and regulate the discharge of spores. The peridial cells are firmly joined by scarf of sutural tip-socles, a particularly tight type of flat articulation, as pictured in FIG. *2*: *14*.

Even more advanced is the articulation of the peridial cells of a balanoid aecium. The peridial cells form long filaments, which are stretched between the apicular cap and aecial bottom. Due to this strained position, the filaments have developed particularly firm double-notched joints (FIG. *2*: *15–17*).

According to the material available, the following types of aecia are clearly distinguishable:

I. AECIOID TYPES

A. *Cupulate* (FIG. *2*: *1*): a cup-shaped aecium, as in Pucciniaceae and Pucciniastraceae. The aeciospores are born in a rounded peridium, dehiscent at apex. The margin is commonly lacerate, spreading or somewhat recurved. The peridial cells are rhomboid and without special articulation. The outer wall of the peridial cell is thicker than the inner one (FIG. *2*: *13*). Examples: *G. libocedri* and *G. nootkatense*. According to Dodge and Buller the spores are shot away from cupulate aecia into the air (**4**, p. 403).

B. *Cylindrate* (FIG. 2: 2): a cupulate aecium with cylindrical peridium, dehiscent at apex. The margin may be firm, lacerate, or fimbriate. The peridial cells are rhomboid, without articulations. In a cylindrate aecium mature, spores are stored until their discharge.[3] Examples: *G. ellisii, G. miyabei, G. cunninghamiana* and *G. cupressi.*

II. PROTUBEROID TYPES

A. *Protuberate* (FIG. 2: 3, 16): a most peculiar type among the aecia of rust fungi. Almost mycelioid peridial cells form a long delicate tube with a short sack-shaped sorus at the apex, like that of *G. transformans.* Firmness is provided by the surrounding host tissue, which forms a long compact protuberance around the lower part of the aecium.

B. *Strobilate* (FIG. 2: 4). There is an observable tendency among protuberoid aecia to form a new strobilate type (*G. biseptatum*), which is functionally similar to the balanate roestelium. (See below.)

III. CORNUOID TYPES

A. *Tubulate* (FIG. 2: 7): in its function similar to the cylindrate aecioid type. It differs from the latter mainly in its more advanced mechanization and more stream-lined structure. The peridial cells of the tubulate aecium are more elongated and provided mostly with a simple, overlapping or scarf articulation. The tubulate aecia are ruptured regularly at the apex and emptied by the wind. Many species belong to the tubulate type: *G. haraeanum* and *G. inconspicuum* are examples. Further development of cornuoid aecia continues from the tubulate forms to the clathrate and cancellate types.

B. *Clathrate* type: a tubulate aecium whose peridium splits into several strips and filaments. In this way mature spores are disseminated by wind during the shortest possible time. Examples: *G. nidus-avis, G. exterum, G. japonicum, B. globosum, G. juniperinum, G. trachysorum.* The clathrate type shows a gradual transition to the highest form of the basculate type.

[3] It would be appropriate to notice here that some forms of *Puccinia graminis* have a somewhat similar evolutionary tendency, in forming long cylindroid aecia in high mountains. The writer collected a considerable number of cylindrical aecia on *Berberis vulgaris* on the Swiss alps (Zermatt) in 1928. Infrequently these types appear on the plains far away from the mountains, and were collected by the writer in 1935 in several places in Estonia and once in Finland. In these places they seem to be unstable and disappear soon. Sometimes the cylindrate types of *Puc. graminis* are described as new species.

C. *Cancellate* type: similar to a tubule, but rupturing laterally. The discharge of spores, therefore, takes place through the peridial lattices. Peridial cells are filamentous and have a firm articulation. Examples: *G. clavariforme, G. corniculans, G. clavipes.* The further development of this trend is accomplished in the balanate type.

IV. ROESTELIOID TYPES

A. *Basculate* (FIG. 2: 5): a roestelioid aecium formed by a short protuberance of host tissue and a moderately long peridial tube. The peridial cells are commonly elongated and rhomboid, with extremely thick inner walls. In the longitudinal direction these cells are held together by *scarf joints* (FIG. 2: 14). The side walls are loosely held together, and easily ruptured. The long chains of peridial cells are stiff rods which curve outwards when dry but close over the mouth of peridium when wet. This observation was made first by Kern in 1910 and was confirmed later by many others.

The alternate closing and opening of the basculum in the presence of moisture or dry air aids in the regulation of discharge of aeciospores. The spores are not immediately released after their maturation, but are gradually pushed through the tube of the protuberance into the spore basket where they are stored until the next favorable period for discharge and distribution.

The writer observed the closing and opening of the peridial strips and rods of apple rusts, *G. juniperinum, G. betheli,* and *G. juniperi-virginianae.* The peridial strand of a basculate aecium is so sensitive to moisture that simply breathing on the open peridium is sufficient to close it immediately. It can be demonstrated also with dried herbarium material, sometimes more than 80 years old.

B. *Balanate* (FIG. 2: 6) (balaniform according to Kern **13**, p. 430). With regard to its mechanism for regulating the discharge of spores, this type can be considered as one of the more advanced forms of aecia. Most peridial cells are long and filamentous with a firm double-notch articulation. The hygroscopic peridial filaments are fixed by both ends and stretched between the apicular cap and the aecial bottom. In dry weather they curve outward, forming numerous cancellate openings for the discharge of spores; when moist, the filaments straighten and close the openings.

Thus the roestelioid sori of *Gymnosporangium* have ceased to be merely a protective organ but serve also to regulate the discharge of spores.

PRELIMINARY PHYLOGENETIC CLASSIFICATION OF GYMNOSPORANGIUM

Kern (**13**, **14**) recognizes 46 species [4] of the genus *Gymnosporangium*, which, however, do not represent equal phylogenetic units. Some of the species which inhabit the ancient genera of Cupressaceae have preserved their archaic traits; some others are rather modern. Arthur (**1**) divided the genus *Gymnosporangium* into two sections: *Gymnotelium* Syd. and *Eugymnosporangium*. H. Sydow, in Ann. Mycol. **19**: 170, 1921, on the contrary, considered *Gymnotelium* to be a new genus.

There is substantial evidence that these two groups, possibly subgenera, are made up of further phylogenetic units. As a result of consultation with Dr. F. D. Kern, the units are arranged as sections I–X, as described below.

In the light of present-day distribution, the provisional sections I–VI of the older group *Gymnotelium* are merely ancient relics which live isolated in the expanding mass of the youthful *Eugymnosporangium*. Although some primitive forms of the subgenus *Gymnotelium* have preserved their archaic characters, none of the existing species can be considered to be the ancestor of the whole genus. All sections are rather parallel-evolving trends which arose from a common ancestral stock (FIG. 1), provisionally called *Proto-Gymnosporangium*.

Preliminary list of sections (I–X) and paragraphs (1–24) of Gymnosporangium

In the following list the species with similar phylogenetic characteristics are grouped into 10 sections and 24 paragraphs. There is morphological evidence that the species in these paragraphs are closely related genetically, although they may have somewhat different evolutionary tendencies. This classification and nomenclature is based upon the diagnoses presented by P. and H. Sydow (**25**), Arthur (**1**) and Kern (**13**). Assumable phylogenetic relationships and observable evolutionary trends in the sequence of these provisionally numbered sections are pictured in FIGS. 1 and 4. Some morphologically intermediate sections between *Gymnotelium* and *Eugymnosporangium* exhibit clear differences in their biological specialization.

A. GYMNOTELIUM, SECT. I–VI.

Telia pulvinate, low, not causing matrical hypertrophy, on Cupressaceae; teliospores 2–5-celled; uredia present or wanting; aecia cupulate, cylindrate or protuberate, on Rosales and Myricales.

[4] According to F. D. Kern (in a letter) who is now preparing material for a monographic treatment, there may be 56 or more species in *Gymnosporangium*.

I. 1. *Nootkatense* on *Chamaecyparis* and Pomaceae.

Uredia present. Telia foliicolous; teliospores 2-celled. Aecia cupulate, aeciospores ellipsoid, wall pale yellow: *G. nootkatense* Arth.

II. 2. *Libocedri* on *Libocedrus* and Pomaceae.

Telia foliicolous; teliospores 2–5-celled; aecia cupulate, aeciospores globoid, wall pale yellow: *G. libocedri* (P. Henn.) Kern.

III. 3. *Ellisii* on *Chamaecyparis thyoides* and Myricaceae.

Telia caulicolous; teliospores 2–5-celled; aecia cylindrate, aeciospores globoid, wall nearly colorless: *G. ellisii* (Berk.) Farl.

IV. 4. *Biseptatae* on *Chamaecyparis thyoides* and Pomaceae.

Telia foliicolous or caulicolous, teliospores 2–6-celled or 2-celled; aecia protuberate or strobilate, the peridial cells very long and narrow, almost hypha-like; aeciospores globoid, wall dark cinnamon-brown: *G. biseptatum* Ellis, *G. transformans* (Ellis) Kern, *G. hyalinum* (Cooke) Kern.

V. 5. *Miyabei* on *Chamaecyparis pisifera* and *Sorbus*.

Telia caulicolous, pulvinate; teliospores 2–3-celled; aecia cylindrate, aeciospores globoid, wall cinnamon-brown: *G. miyabei* Yamada & Miyabe.

VI. 6. *Cupressi* on *Cupressus* and *Pyrus*.

Telia caulicolous, teliospores 2-celled; aecia cylindrate, aeciospores ellipsoid, wall light chestnut-brown: *G. cunninghamiana* Barcl., *G. cupressi* Long & Goodd.

B. EUGYMNOSPORANGIUM, SECT. VII–X.

Telia hemispheric, tongue-shaped, otherwise sculptured, or causing hypertrophy, galls, witches' brooms, etc., exclusively on *Juniperus;* teliospores 2-celled, except sect. *Speciosae*. Uredia wanting. Aecia tubulate or roestelioid, mainly on Pomaceae.

VII. 7. *Speciosae* on *Juniperus* sect. Sabina and possibly on Oxycedrus.

Telia caulicolous; teliospores 2- or 3-celled. Aecia tubulate. Aeciospores globoid, wall pale yellow, on Hydrangeaceae: *Fendlera* and *Philadelphus: G. speciosum* Peck.

VIII. 8. *Clavipes* on *Junip.* sect. *Oxycedrus* and *Sabina*.

Teliospores 2-celled, pedicels carrotiform, very long. Aecia tubulate; aeciospores globoid with apple-yellow walls. *G. clavipes* C. & P., *G. inconspicuum* Kern.

IX. 9. *Multiporae* on *Junip.* sect. *Sabina.*

Teliospores 2-celled, cinnamon-brown, pores 5–7 in each cell, large, scattered. Otherwise with primitive characteristics. *G. multiporum* Kern.

X. *Eutelium* on *Juniperus* sect. *Oxycedrus* or *Sabina.*

True eutelioid forms with cornuoid and roestelioid aecia, descending very likely from a common ancestral stock on *Proto-Juniperus.*

10. *Communis* on *J. communis* and related species. This is the score of juniper rusts, most common in the Old World. The representatives of these groups have some striking similarities in their basic characteristics and have almost the same host range. See also Nos. 18 and 19. *G. amelanchieridis* (C.D.C.) Ed. Fisch., *G. aurantiacum* Chev., *G. torminali-juniperinum* Ed. Fisch., *G. davisii* Kern.

11. *Haraeanum* on *J.* sect. *Sabina.* Telia foliicolous, teliospores all ellipsoid. Aecia, tubulate, peridial cells compact-overlapping, with verrucose or spinulose walls. *G. haraeanum* Syd., *G. Harknessianum* (E. & E.) Kern, *G. exiguum* Kern.

12. *Yamadae* on *J.* sect. *Sabina: G. yamadae* Miyabe from Japan; see diagnosis.

13. *Confusum* on *J.* sect. *Sabina.* Telia caulicolous, causing hypertrophy, conic, on fusiform swellings. Aecia tubulate or clathrate, peridial cells rugose. *G. confusum* Plowr.

14. *Japonicum* on *J.* sect. *Sabina: G. japonicum* Syd. (see diagnosis).

15. *Nidus-avis* on *J.* sect. *Sabina.* Telia caulicolous, often forming witch's brooms. Aecia cornuoid, peridial cells rugose: *G. kernianum* Bethel, *G. nidus-avis* Thaxt.

16. *Trachysorum* on *J.* sect. *Sabina: G. trachysorum* Kern. See diagnosis.

17. *Exterum* on *J.* sect. *Sabina,* and *Rosales* (Portheranthus): *G. exterum* A. & K. See diagnosis.

18. *Clavariaeforme* on *J.* sect. *Oxycedrus.* Differs from paragraph 10 in its lanceolate teliospores and long verrucose peridial cells. *G. clavariaeforme* (Jacq.) DC.

19. *Juniperinum* on *J. communis* and related species. Differs from paragraph 9 by its advanced aecia and telia, but has a number of similar characteristics. *G. juniperinum* (L.) Mart.

20. *Bermudiani* on *J.* sect. *Sabina.* Both telia and aecia on *Juniperus,* otherwise with advanced characteristics (see Kern, **13**, pp. 408–409). The formation of aecia on the telial host is very likely a secondary change in the life cycle of *Gymnosporangium,* exemplified by several

analogous cases among Melampsoraceae (Leppik, **22**, f. 4, p. 58). *G. bermudianum* (Farlow) Earle.

21. *Corniculans* on *J.* sect. *Sabina.* Telia caulicolous, causing galls. Aecia horn-shaped, mostly cancellate, with peridial cap, dehiscent by side slits. Peridial cells rhomboid or broadly lanceolate, remaining straight when wet, cell walls moderately verrucose. *G. corniculans* Kern., *G. nelsoni* Arth.

22. *Globosae* on *J.* sect. *Sabina.* Telia on galls or gall-like excrescences. Teliospores ellipsoid, with long pedicel. Peridial cells rugose with ridges. *G. globosum* Earl., *G. betheli* Kern.

23. *Juniperi-virginianae: G. juniperi-virginianae* Schw. See diagnosis.

24. *Sabinae* on *J.* sect. *Sabina.* With balanoid aecia and most advanced telia. *G. sabinae* (Dicks.) Wint.

BIOLOGICAL SPECIALIZATION OF GYMNOSPORANGIUM

The genus *Gymnosporangium* on the Cupressaceae and Pomaceae is common in the temperate zone of the northern hemisphere. It extends to the verge of the tropics only in some few places at high altitudes. In spite of the cosmopolitan character of the Cupressaceae, only the northern genera of this family are inhabited by rusts. This fact indicates the possible origin of the genus *Gymnosporangium* from the common stock of aecioid conifer rust, which is distributed exclusively in the northern hemisphere.

A coordinated picture of the biological specialization of the genus *Gymnosporangium* is delineated in FIG. 3. These sketches do not pretend, however, to be complete. According to this scheme the phylogenetically older sections of *Gymnotelium* (thick lines in FIG. 3) inhabit many older genera of Cupressaceae, and *Eugymnosporangium* (thin lines in FIG. 3) is specialized to live exclusively on *Juniperus.* As might be expected, the number of sections in this old group is relatively high (**6**), but the number of species in each section and the frequency of distribution of each species is drastically reduced.

The group *Eugymnosporangium* is evidently of later origin. It contains fewer sections than *Gymnotelium,* but has considerably more species in each section. These sections are rich in modern types which have a wide distribution in both the Old and the New World.

Still younger relatives of the genus *Gymnosporangium* have restrained their life cycle exclusively to some genera of Pomaceae and are separated by Dietel (**5**, p. 77) into a new genus *Coleopuccinia* Patouillard (FIG. 3).

The biological specialization of the genus *Gymnosporangium* has a marked similarity with the host range of *Melampsora* on the Abietaceae (see **22**, p. 58, f. 3). The genus *Melampsora* has its antique forms on *Abies*, a vigorous spread on *Larix* and the Salicales, and modern types on various higher angiosperms. This correlation of the biological spe-

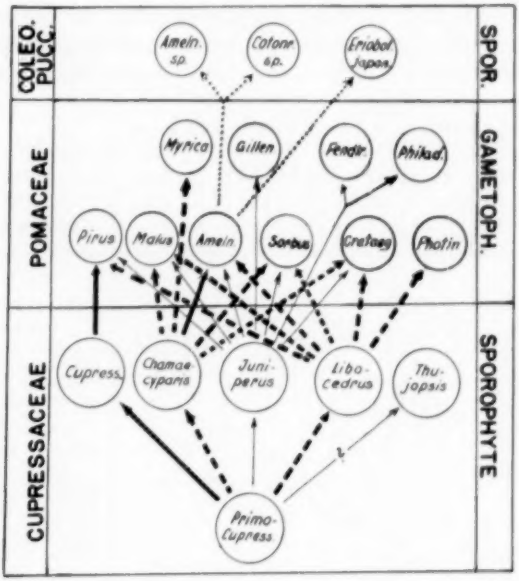


FIG. 3. Historical development of the biological specialization of the genus *Gymnosporangium*. Thick lines indicate *Gymnotelium*, thin lines *Eugymnosporangium*, dotted lines *Coleopuccinia*.

cialization of *Gymnosporangium* and *Melampsora* points to a similar history of these distantly related rust genera and their main host groups, the Cupressaceae and Abietaceae.

SOME OBSERVABLE EVOLUTIONARY TENDENCIES OF GYMNOSPORANGIUM

According to Kern (**13**, p. 408), the possession of the cupulate-type of aecium, foliicolous telia, and the presence of uredia are characteristics of the most ancient species of *Gymnosporangium*. Generally the small foliicolous forms which cause no hypertrophy may be considered as primitive, and the species which occur on the branches, with fusiform swellings and gall-like outgrowths, are relatively specialized.

An analogous interpretation of these morphological characteristics is implied also by Arthur (1) in his sequence of sections and numbering of species. Such grouping of sections and species also corresponds generally to the sequence of biological specialization (see above).

There is also an observable tendency in the evolution of teliospores. Except *G. nootkatense*, most primitive sections of the group *Gymnotelium* are in possession of many-celled teliospores, while the number of cells in the same species is not constant. Several parallel evolving sections have the same tendency to reduce the cell number of their teliospores

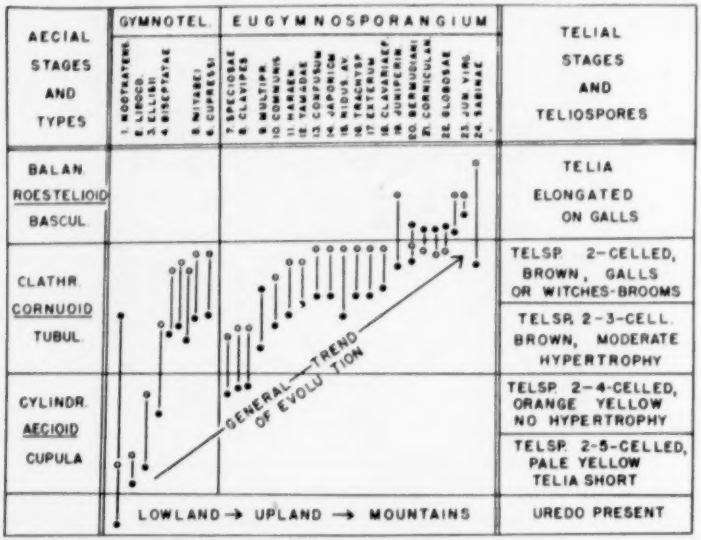


FIG. 4. Paragraphs 1-24 of the genus *Gymnosporangium*, arranged according to primitive and specialized characters of their aecial (white circles) and telial and uredial stages (black dots).

to two, seldom to one (*G. tsingchenensis* from China). Higher sections of the group *Eugymnosporangium* have exclusively 2-celled teliospores.

Some general conclusions concerning relative phylogenetic ages of definite groups can be deduced only from the comparative study of all main characteristics and the sequence of biological specialization (FIG. 4).

In FIG. 4 are indicated six subsequent stages of the evolutionary development of telia, teliospores, and aeciospores, as follows: 1. The presence of urediniospores. 2. Telia short, teliospores many celled, the

number of cells not fixed, mostly 2–5; walls of aeciospores pale yellow. 3. Teliospores 2–4-celled; no hypertrophy; aeciospores orange-yellow. 4. Teliospores 2- or 3-celled; moderate hypertrophy; aeciospores brown. 5. Teliospores 2-celled, on small galls or witches' brooms. 6. Telia elongated, on galls. *G. nootkatense* is an exception. It has preserved its ancestral uredia and cupulate aecia, and is, therefore, considered to be a connecting link between *Gymnosporangium* and aecioid rusts, in spite of its 2-celled specialized teliospores (Jackson **12**, p. 73; Gäumann **10**, p. 321; Buller **4**, p. 411).

TABLE I

MAIN STAGES AND TRENDS IN THE EVOLUTION OF AECIAL TYPES OF GYMNOSPORANGIUM

Stages	Gymnotelium	Eugymnosporangium	
Roestelioid		Basculate FIG. 2: *5*	Balanate FIG. 2: *6*
Cornuoid	Strobilate FIG. 2: *4*	Clathrate	Cancellate
	Protuberate FIG. 2: *3*	Tubulate (conic, cornute) FIG. 2: 7	
Aecioid	Cylindrate FIG. 2: *1*		
	Cupulate FIG. 2: *1*		

Even more clear is the evolution of aecia (FIG. 2: *1–8*, TABLE I). Beginning from the simple cupulate type, many varieties and several parallel trends lead to the better regulation of the discharge of spores, as described above.

Three successive hologenetic stages of four different phylogenetic trends in the aecial development of *Gymnosporangium* are outlined in TABLE I.

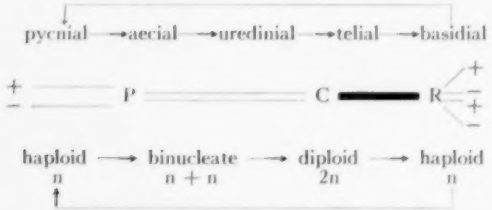
Among sections of the subgenus *Gymnotelium*, two phylogenetic trends are clearly distinguishable. One of these trends is expressed in the further development of a simple cupuloid aecium as exemplified in the sections *Nootkatense*, *Libocedri* and *Ellisii* to a cylindrate type. The close phylogenetic kinship of these sections is clearly expressed in the identical structure and similarly abutted peridial cells (FIG. 2: *9*,

10, 13). Another trend is the development of mycelioid peridial cells with overlapping articulation (FIG. *2*: *16*). This trend has led to the protuberoid aecium and has had a further tendency toward the strobilate type (FIG. *2*: *3, 4, 16*).

The group *Eugymnosporangium* is represented by many more aecial types. A great many species of this subgenus are in possession of tubulate, cornute, conic, or otherwise elongated aecia of various structure (FIG. *2*: *7, 8, 11, 12, 14, 15*). Two evolutionary trends are distinguishable among these types. One leads through clathrate-type to the basculum (FIG. *2*: *5*), another through cancellate type to balanum (FIG. *2*: *6*).

TABLE II

NORMAL LIFE CYCLE OF AECIOID RUST FUNGI. IN THE UPPER ROW ARE INDICATED THE SPORULATING STAGES; IN THE MIDDLE ROW THE CYTOLOGICAL CHANGE; IN THE LOWER ROW THE ALTERNATING NUCLEAR PHASES



Abbreviations: P = Plasmogamy; C = Caryogamy; R = Reduction Division (meiosis); n = number of chromosomes in nucleous.

A similar correlation between the evolutionary sequences of rusts and their host plants has been recently established by Savile (**24**) in the microcyclic rusts on Saxifragaceae.

ORIGIN OF THE GENUS GYMNOSPORANGIUM

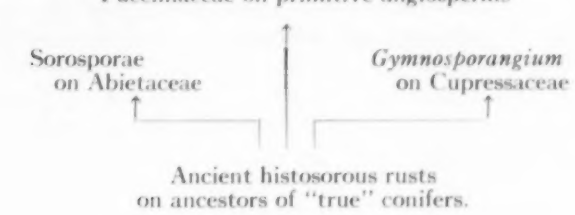
There is some evidence now that all "true" aecioid rusts [5] on conifers and angiosperms, including the genus *Gymnosporangium*, have a distant genetic relationship with one another and presumably descend from a common ancestral stock. In discussing this question, more emphasis must be placed on the fact that all these fungi have a common sequence of spore forms (TABLE II).

[5] This classification is a preliminary one, based upon the material available to the writer. It is, however, useful for the present consideration of the aecial stages and hologenetic levels as pictured in FIG. 4.

Knowledge of this genetic relationship makes the present attempt to derive the genus *Gymnosporangium* and related groups from the common stock of aecidioid conifer rusts highly reliable. It is an obvious advantage to this study that the departure of a new evolutionary trend among Uredinales and a new phylogenetic branch of cedar and cypress rusts is clearly marked by a new, reverse sequence of hosts, as indicated in the beginning of this article. This exchange of hosts might have occurred when the "true" conifers split into the recent families Abietaceae, Cupressaceae and Taxodiaceae, but before the Cupressaceae had divided into present genera. These circumstances enable us to connect the origin and evolution of the genus *Gymnosporangium* with the phylogeny of conifers and to fix it with geological history (**22**, **23**). The age of the genus *Gymnosporangium* according to this theory could be somewhat shorter, but not longer than the history of Cupressaceae.

PROTO-GYMNOSPORANGIUM

Subsequent segregation of recent conifer families, Abietaceae and Cupressaceae, from their Mesozoic ancestors has presumably also caused the splitting of the ancient histosorous rusts into several new groups, as follows:



Large upland areas might have been already settled at that time by more specialized groups of conifers and angiosperms, providing a new ground for aecioid rusts. Pucciniaceae settled themselves according to this hypothesis together with expanding angiosperms in newly formed grassland areas, but the genus *Gymnosporangium* started to move with Cupressaceae toward mountains.

The climatic change from the humid plains to the arid uplands is most markedly demonstrated in the advanced soral structure of the genus *Gymnosporangium*. Both the jelly-like telia and the well-developed peridial cover of the aecia provide an adequate protection of immature

spores against drought (**22**, pp. 50–53). It would be an improbable assumption that such protective devices could have developed in a moist climate. The further movement of some groups of the Cupressaceae from uplands to the mountains is reflected in the evolution of roestelioid aecia.

CONCLUSIONS

A comparative phylogenetic study of the genus *Gymnosporangium* and its host plants reveals some new facts and circumstances concerning the historical development of both partners. Morphological structure of aecia, presence of uredinia in an ancient form, the successive seriation of biological specialization on Cupressaceae and the observable evolutionary tendencies indicate a distant relationship of the genus *Gymnosporangium* with "true" aecioid rusts on conifers. There is an obvious parallelism between the evolution of the genus *Gymnosporangium* and its main hosts of the cypress family.

Several protective devices of telia and regulating mechanisms of aecia permit some reliable conclusions concerning the climatic conditions of the past, necessary for the development for such special soral types. The observable evolutionary tendencies of these climatic types point to the possible change in the weather conditions during their development. In the abundance and continuity of new types and specialized forms of the genus *Gymnosporangium* is reflected a vigorous push of plant life from a humid subtropical zone toward more arid and cooler northern areas and from the lowlands to the mountains.

A serial arrangement of the proposed subdivisions of *Gymnosporangium* in FIG. 4 makes it possible to establish approximately the relative age of these groups.

ACKNOWLEDGMENTS

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amount of the material used for this study has been collected by Mag. Alex Kivilaan from Shenandoah, Iowa.

Professor Theodore Hong of Augustana College read my manuscript and made some grammatical corrections.

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SUPPLEMENTARY DEVELOPMENTAL STAGES OF BASIDIOBOLUS RANARUM AND BASIDIOBOLUS HAPTOSPORUS

CHARLES DRECHSLER [1]

(WITH 5 FIGURES)

During the 70 years that have elapsed since *Basidiobolus ranarum* was described by Eidam (**7**) as the type of a new genus of the Entomophthoraceae this fungus has retained widespread interest by virtue of outstanding peculiarities in its development and morphology. The rocket-like action by which the expanded distal portion of its conidiophore propels the globose conidium (**10**) would seem different in principle from the various mechanisms for forcible spore discharge operative in related genera and in other groups of fungi. Its curious sexual reproduction, wherein a preliminary division of the nuclei of adjacent conjugating hyphal segments takes place simultaneously in paired juxtaposed protuberances, has deservedly been set forth in many text-books on mycology. Yet somehow it has not become equally well known that the nuclei of *B. ranarum*, as also those of congeneric species, are for the most part readily visible in unstained living material, being obscured from view only in thick structures, such as young zygospores and large globose conidia, where they lie deeply imbedded in protoplasm of dense, coarsely granular texture. Although the visibility of its nuclei and the ready conjugation of its paired hyphal segments in a period of approximately 2 hours would seem exceptional features that might make *B. ranarum* very desirable for purposes of instruction, the fungus has not been a familiar object in American laboratories. As far as can be determined from the literature it was obtained from American materials only twice during the 65 years directly following its description: Thaxter (**15**) having grown it on frog excrement procured by filtering out sediment from water in which frogs were kept, and Olive (**13**) having brought it into pure culture by allowing conidia formed on contents removed from the intestine of a frog to be shot on to small cubes of sterilized bread. The culture of *B. ranarum* used by Couch (**1**) in experi-

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ments on sexual reactions between entomophthoraceous forms would seem to have originated abroad, having apparently been supplied earlier from a foreign culture collection.

An aversion for killing and dismembering largish animals other than insects may have deterred many American mycologists from acquiring material of *Basidiobolus ranarum* firsthand. Eidam's stern procedure in slaughtering dozens of frogs at a time in order to obtain quickly a generous supply of their intestinal and stomach contents can not be considered at all alluring, and in suburban areas where amphibians seem generally scarce would be against the public interest and difficult to carry out. Fortunately *Basidiobolus* isolations can be obtained readily from much smaller quantities of frog excrement than Eidam believed necessary, so that all destruction of the animals can be obviated. A captured frog confined in a clean glass jar containing 25 to 50 cc of distilled water will commonly void sufficient excrement in 10 to 15 hours, and can then be returned unharmed to its habitat. The excrement, though somewhat gelatinous, may be collected conveniently by passing the liquid contents of the jar through a small paper filter that has been snugly nested in firmly packed absorbent cotton or absorbent paper. If the filter is then flattened out while still moist, and portions of it, soiled with excrement, are affixed, soiled side downward, to the ceiling of sterile Petri dishes containing sterile maize-meal agar, numerous small *Basidiobolus* mycelia, each coming from a separate conidium, will usually appear 24 to 48 hours later in scattered positions on the agar surface. Even the very scanty excrement voided in 15 hours by individual frogs belonging to *Pseudacris nigrita feriarum* (Baird), and weighing only about 5 grams, always yielded many *Basidiobolus* mycelia when brought into a paper canopy over a Petri plate of maize-meal agar. By transferring the small mycelia to sterile agar slants pure cultures were usually obtained at once.

In a collection of 57 *Basidiobolus* cultures obtained from excrement of 15 frogs captured in 4 locations near College Park, Maryland, on June 29 and July 23, 1955, two species were readily distinguished. All isolations, 18 in number, derived from excrement of 2 frogs (*Rana clamitans* Latreille) captured on the earlier date emitted the musty benzene-hexachloride odor familiar in species of *Streptomyces*, and promptly gave rise to numerous propulsive conidiophores as well as to an abundance of zygospores surrounded individually by a wall of undulate outer profile. Among the 39 cultures isolated from excrement of 13 frogs [2] captured on the later date only 2 emitted the musty *Strepto-*

[2] Of these animals 3 belonged to *Rana clamitans* and 10 to *Pseudacris nigrita feriarum*.

myces-like odor and produced zygospores of undulate profile. The remaining 37 cultures emitted no musty odor, and promptly formed numerous smooth zygospores while at the same time giving rise to large numbers of phototropic conidiophores that shot off globose conidia. After 10 days they showed a layer of white aerial mycelium and could thereby be definitely referred to my *B. meristosporus* (**6**). It is of some moment that the 2 cultures of the odorous *Basidiobolus* obtained from the second group of frogs came from separate individual animals, each of which yielded, besides, several cultures of *B. meristosporus*.

As no European writings on *Basidiobolus ranarum* suggested any likelihood that 2 members of the genus might occur in frog excrement, the isolation of 2 species from the excrement voided by individual frogs in the course of one day was wholly unexpected. That the 2 species included *B. meristosporus* was especially contrary to expectations, for though this fungus, through its ready production of elongated adhesive conidia and sporangia, appeared well adapted to colonize the digestive organs of amphibians, it had previously become known to me only from plant detritus collected in Florida, never having developed in cultures prepared with decaying vegetable remains gathered in Maryland and Virginia. However, when Petri plates of maize-meal agar were canopied on July 26, August 1, and August 10, 1955, with leaf mold newly collected near College Park—finely divided forest detritus being affixed to the lids sometimes by means of agar and at other times by means of moist filter paper—many isolations of *B. meristosporus* were obtained. In these canopied plate cultures neither of the 2 species I had previously obtained from plant detritus collected in Maryland and Virginia (**3, 4, 6**) came to light. The divergent results have their explanation in the different temperature adaptations of the fungi here concerned. All the earlier work with decaying material from Maryland and Virginia was performed in winter at room temperatures near 20° C, so that the cultures prepared with detritus newly gathered outdoors favored the development of the 2 species, *B. haptosporus* and the odorous form, in which zygospore germination, growth, and reproduction proceed well at such temperatures. The Petri plate cultures canopied with excrement of the 2 frogs captured on June 29, likewise were incubated at temperatures near 20° C, and thus permitted development of the odorous species after its vegetative cells had given rise to phototropic conidiophores and globose conidia. For lack of cooler chambers, the plate cultures canopied with excrement of the 13 frogs captured on July 23, as also the plate cultures canopied with newly collected leaf mold on July 26, August 1, and August 10, were incubated in a basement room at 27° C—a tem-

perature less favorable for the odorous fungus than for the somewhat more thermophilic *B. meristosporus*. Besides, *B. meristosporus* must have been present more abundantly in the materials used for canopying cultures in July and August, as the preceding weeks of hot mid-summer weather could hardly have failed to bring about greater development of the more thermophilic species, and correspondingly more plenteous ingestion of its adhesive conidia by amphibians.

MORPHOLOGICAL FEATURES OF THE ODOROUS FUNGUS AND ITS PRESUMPTIVE IDENTITY WITH BASIDIOBOLUS RANARUM

All the *Basidiobolus* isolations producing zygospores of undulate profile that were obtained from frog excrement seem clearly referable to the same species as the congeneric isolations of like sculpture which I reported earlier (**4**, **5**, **6**) to have been procured from plant detritus collected in New Hampshire, Pennsylvania, Delaware, Maryland, Virginia, North Carolina, and Louisiana. More recently, similar and manifestly conspecific isolations have been obtained also from plant detritus gathered during the third week in November, 1954, in Chicago, Illinois; in Fort Wayne, Indiana; and near Park Falls, Butternut, Mellen, and Grandview in northern Wisconsin. Whether obtained from frog excrement or from plant detritus, the *Basidiobolus* forming undulate zygospores gives off a distinctive benzene-hexachloride odor similar to that emitted by many species of *Streptomyces*. During the period when the mycelium is actively growing, this odor, as a rule, is given off strongly. Only one among more than 150 conspecific isolations in my collection produced the odor so faintly that it might readily have remained undetected. A culture (ATCC*11230*) maintained at the American Type Culture Collection, Washington, D. C., under the binomial *B. ranarum*, which presumably originated in the Old World, also was found to give off a strong *Streptomyces*-like odor. As this culture apparently lacks both sexual and asexual reproduction, and as its hyphal segments often show a rather pronounced median distention, it was held earlier (**5**) to represent a species alien to the odorous cultures isolated in the United States. This opinion may have been incorrect, for if the culture should have lost its reproductive capacities, possibly from long continued propagation on artificial substrata, pronounced modification in outward shape of hyphal segments might have ensued as a direct consequence. When reproduction is temporarily inhibited in my cultures because of unsuitably high temperatures, the hyphae and their segments often show conspicuous modifications. In any case the sterile culture gave little help toward determining the relationship of my odorous isolations to *B.*

ranarum. Eidam's original account of that species makes no mention of any distinctive odor; nor, apparently, is any odor discussed by later European investigators that have dealt with *B. ranarum*.

In its earlier, purely vegetative growth on a transparent substratum the odorous *Basidiobolus* does not differ markedly from *B. meristosporus* and *B. haptosporus*. The main hyphae growing out radially at the margin of a mycelium that is expanding unimpeded in a Petri plate of maize-meal agar commonly measure about 10 μ in width (FIG. 1, *A*, *B*). As the terminal segment elongates it divides repeatedly in forming one penultimate segment after another. Rather often the segments some distance from the mycelial forefront are noticeably wider than those at the periphery. In a mycelium that has originated from a globose conidium the portions of hyphae near the empty conidial envelope may measure 15 to 20 μ in diameter. Although the mycelium produced in maize-meal agar of low nutrient content is generally robust, it may yet be hardly visible to the naked eye except by reflected light. On maize-meal agar with much fine maize-meal in suspension, as also on Lima-bean agar, a substratum even richer in nutrients, the hyphae are extended in closer arrangement, so that the mycelium is revealed more clearly to the naked eye, usually as a smooth colorless layer of somewhat cartilaginous appearance.

At temperatures near 20° C a mycelium of the odorous *Basidiobolus*, on attaining a diameter of several millimeters, usually begins to produce both conidia and zygospores. As it continues to expand, asexual and sexual reproduction proceed concurrently, some hyphal segments sending up individually a stout conidiophore (FIG. 1, *C*), while others nearby conjugate in pairs. Owing to the strong phototropism of the conidiophores, the single globose conidia they shoot off fall in scattered positions beyond the mycelial forefront on the expanse of unoccupied substratum extending toward the main source of light. Many of the scattered conidia may each give rise directly to a stout phototropic conidiophore that soon shoots off a single globose secondary conidium to a position farther toward the light. Other conidia germinate vegetatively to form small subsidiary mycelia which give rise to plural phototropic conidiophores, each of which likewise shoots off a single globose conidium toward the light. In Petri plate cultures, therefore, the odorous fungus, much like *B. meristosporus*, spreads rapidly and disconnectedly over the areas lying toward the main source of light, while its advance in other directions proceeds more slowly and uniformly through elongation of the hyphae at the mycelial forefront.

In all my isolations of the odorous *Basidiobolus* the globose conidia

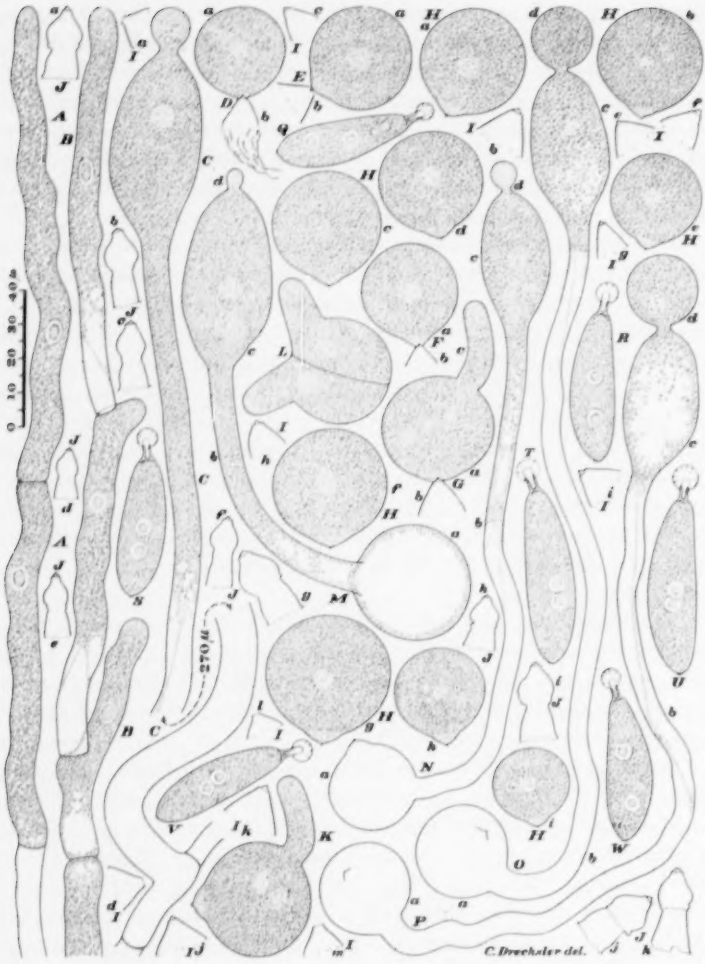

FIG. 1, *A–W*.

FIG. 1. *Basidiobolus ranarum* as found in maize-meal-agar plate cultures, × 500. *A, B.* Distal portions of elongating hyphae at margin of a growing mycelium. *C.* Young conidiophore that grew out from a submerged hyphal segment (a portion 270 μ long being omitted). *D–G.* Globose conidia, *a,* with empty membrane of propulsive enlargement, *b;* the conidium in *G* is extending a germ tube, *c. H.* Detached globose conidia, *a–i. I.* Detached conical pieces of membrane, *a–m,* from tips of propulsive enlargements. *J.* Empty membranes, *a–k,* of propulsive

were shot off forcibly after the manner set forth in Eidam's original account of *B. ranarum*. As Nowak (**12**) has contended that in *B. ranarum* the globose conidia are not forcibly discharged, occasion may be taken to emphasize that none of my isolations have under conditions not obviously unfavorable failed to show energetic conidial propulsion. The discharge mechanism, of course, fails to operate in material mounted in water under a cover glass, the fully developed conidium (FIG. 1, *D, a*) in moist preparations remaining attached to the empty conidiophore, which except for a widened distal portion (FIG. 1, *D, b*) soon collapses and evanesces. In some instances a conidium (FIG. 1, *E–G: a*) after being shot off forcibly has attached to its base an empty funnel-shaped membrane (FIG. 1, *E–G: b*). More often, however, the conidium at the end of its flight (FIG. 1, *H, a–i*) is found unencumbered by any membranous attachment. Empty pieces of membrane, some of them conical or funnel-shaped (FIG. 1, *I, a–m*) and others of the curious tower-and-cupola design (FIG. 1, *J, a–k*) made familiar in Eidam's illustrations, become strewn about haphazardly on the substratum. Lying on a moist agar surface many globose conidia (FIG. 1, *G, a; K*) germinate by putting forth a single germ hypha (FIG. 1, *G, c*). A few undergo a division and then extend a germ tube from each of the resulting cells (FIG. 1, *L*). In many instances where a conidium (FIG. 1, *M–P: a*) has put forth a single germ hypha, the hypha (FIG. 1, *M–P: b*) grows upward into the air to give rise on the tip of a propulsive enlargement (FIG. 1, *M–P: c*) to a secondary globose conidium (FIG. 1, *M–P: d*). Such secondary conidia and also all globose conidia of higher orders formed through continued repetitional development, have in my isolations been shot off forcibly, much like primary conidia borne on conidiophores arising from hyphal segments.

Like the hyphal segments from which they are derived, the primary conidia of the odorous *Basidiobolus* vary moderately in size. The globose spores shot off from vigorous young mycelia in maize-meal-agar plate cultures range in length and width between 25 and 40 μ, the averages of the two dimensions in material from separate Petri dishes falling often between 30 and 33 μ. On rich Lima-bean agar larger spores are formed, some primary conidia produced on this medium attaining lengths and widths between 40 and 48 μ, thereby equalling

enlargements. *K*. Detached globose conidium germinating. *L*. Globose conidium extending a germ tube from each of its 2 cells. *M–P*. Four globose conidia, *a*, that have each extended a broad conidiophore, *b*, bearing a propulsive enlargement, *c*, surmounted by a young globose secondary conidium, *d*. *Q–W*. Detached binucleated adhesive conidia.

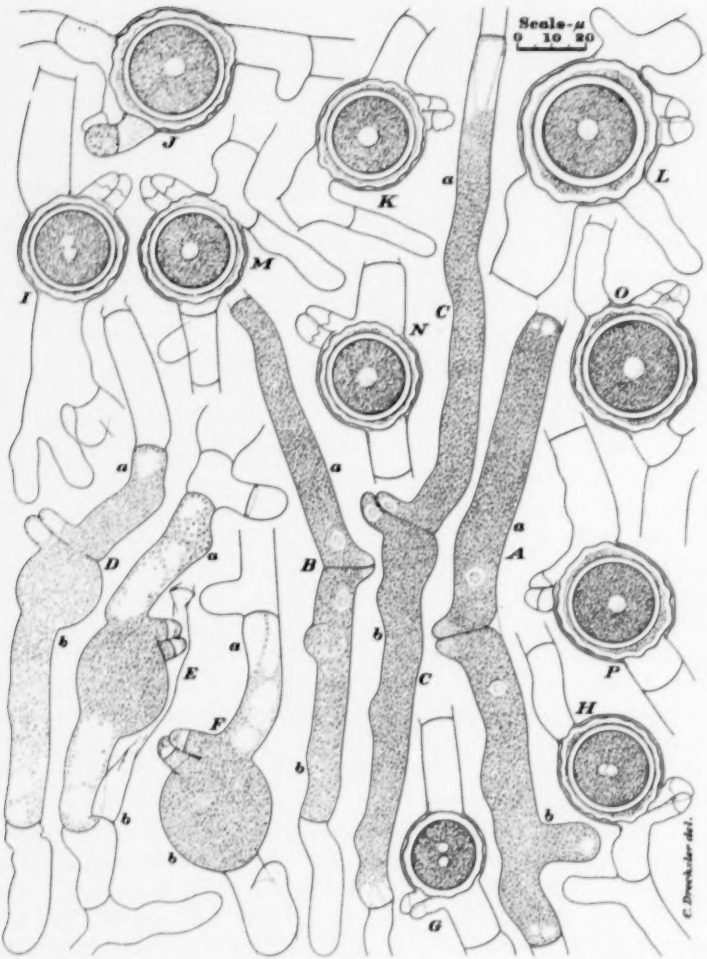

FIG. 2, *A–P*.

FIG. 2. ***Basidiobolus ranarum*** as found in maize-meal-agar plate cultures, × 500. *A–C*. Three young sexual reproductive units, each consisting of 2 adjacent hyphal segments, *a* and *b*, that have put forth apposed protuberances; in *A* and *B* the cell nuclei are moving toward the protuberances, in *C* they have moved into the protuberances. *D–F*. Sexual reproductive units in each of which one hyphal segment, *a*, is supplying protoplasm for the formation of a zygospore in an adjacent segment, *b*. *G*. Mature zygospore showing no separation of wall into 2 layers.

the maximum measurements indicated in the dimensional ranges (23 to 48 μ for length, 21 to 46 μ for width) that Eidam ascribed to the conidia of *B. ranarum*. It is worthy of note that the several full grown conidia figured by Eidam would seem from the indicated scales of magnification to vary from 28 to 33 μ in length, and thus are generally comparable in size with the dozen globose conidia (FIG. 1, *D–F: a; H, a–i*) figured herein as being tolerably representative of the odorous fungus. These dozen spores and, indeed, all other structures of the odorous species shown in FIGS. 1–3, were drawn from specimens taken from maize-meal-agar plate cultures of an isolation obtained from leaf mold gathered in oak woods near Farmer, North Carolina, late in December, 1951. In view of the rather wide range of variations commonly observable in a single culture, the differences between separate isolations of the odorous species, whether originating from plant detritus or from frog excrement, have not seemed very pronounced.

As has been mentioned, sexual reproduction in the odorous *Basidiobolus* usually proceeds concurrently with asexual reproduction after a young mycelium has attained a diameter of several millimeters. Conjugation always takes place between adjacent hyphal segments (FIG. 2, *A–C: a, b*) and is accomplished in the manner described in Eidam's account of *B. ranarum* through production of juxtaposed protuberances, division of the nuclei in the protuberances, partial solution of the separating wall, and movement of the protoplasm, together with a nucleus, from one of the gametes (FIG. 2, *D–F: a*) into the other (FIG. 2, *D–F: b*). The fusion cell thus formed soon lays down a thick wall with a smooth circular inner contour and an undulated outer contour (FIG. 2, *G;* FIG. 3, *E, F*). Fully mature zygospores of moderate size often show a somewhat thicker wall in which 2 layers, separated here and there, can be distinguished. The largest zygospores commonly show during the resting period a wall composed of a smooth inner layer and an undulated outer layer (FIG. 2, *H–P*). Although the inner contour of the outer layer is usually rather indistinct, the 2 layers seem discrete all around, and in some regions are separated by spaces 1 to 2 μ wide. Some of the wider interstitial pockets seem filled to a greater or lesser extent with granular material.

In respect to size the zygospores of the odorous *Basidiobolus* correspond well with those of *B. ranarum*, the dozen specimens figured herein (FIG. 2, *G–P;* FIG. 3, *E, F*) showing approximately the same range in

H–P. Mature zygospores showing separation of wall into an undulate outer layer and a smoothly spherical inner layer; in *J* the distal segment of one protuberance appears filled with living protoplasm.

diameter—23 to 43 μ—that Eidam ascribed to the zygospores of his frog-inhabiting species. The odorous fungus corresponds well also with the description of *B. ranarum* in the undulate contour of the wall surrounding its zygospore. While Eidam (**7**: 221) noted very briefly that the zygospores he observed developing in nutrient solutions began to show stratification of the endosporium as they matured into resting spores, he gave no details on the nature of the stratification. The peripheral markings in his relevant illustrations (**7**: Pl. 12, Figs. 7–9, 12–14), like the similar markings in a figure of *B. ranarum* given by Thaxter (**15**: Fig. 413), seem more expressive of the undulated appearance of the zygospore wall than of its structural make-up, which probably was not well revealed under the microscopes then in use. Subsequently Fairchild, who presumably employed a European culture in the investigations he carried out at Bonn, Germany, on nuclear division and fertilization in *B. ranarum*, supplied figures (**8**: Pl. 14, Figs. 15, 16) showing two thick layers in the zygospore wall proper, and partial separation of these layers. These figures present much the appearance usual in medium-sized zygospores produced by the odorous American species, though in unstained living material of my fungus it has not been easy to distinguish clearly the additional thin outer layer which Fairchild recognized as the original delimiting membrane secreted by the young zygospore.

During their resting period some zygospores of the odorous *Basidiobolus* have a strongly globuliferous internal structure, while others are largely filled with cytoplasm of uniformly coarse texture. Among zygospores of the latter category the larger number display near the center a single globose, somewhat lustrous body (FIG. 2, *J–P*; FIG. 3, *F*) corresponding in size and shape to the fusion nucleus shown in Fairchild's figures illustrating rather old zygospores of *B. ranarum*, while a smaller number display two such bodies (FIG. 2, *G–I*, FIG. 3, *E*). Two lustrous bodies, or nuclei, are commonly visible in zygospores which from their smoothly spherical shape and thin wall (FIG. 3, *G, H*) are evidently ready to germinate. After a protuberance from the globose zygospore (FIG. 3, *I*) has broken through the enveloping membrane to push forth externally, the 2 nuclei can be observed moving forward in the elongating germ hypha (FIG. 3, *J, K*) separated from each other by an interval of 1 to 6 μ. As a rule the separation is no less evident in short germ hyphae than in long ones. This somewhat aloof companionship merits notice because the 2 nuclei in the germinating zygospore of *B. ranarum*, according to Eidam (**7**: 229, Pl. 12, Figs. 19, 20) regularly emerge from the spore envelope in intimate contact

with one another, and remain in intimate contact as they move forward until the germ hypha has attained sufficient length to become divided by cross-walls. Since the 2 nuclei shown by Eidam (**7**: Pl. 9, Fig. 14) in a conidiophorous hypha arising from a globose conidium produced through germination of a zygospore, appear separated by an interval of nearly 2 μ, it would seem that at least the positional relationship later prevailing in *B. ranarum* is approximately as in the odorous fungus.

Eidam observed that the binucleated condition in germ hypha extended from a zygospore of *Basidiobolus ranarum* is always terminated when mycelial development ensues. In the odorous fungus similarly the uninucleated condition is always restored whenever the vegetative state is resumed. Under natural conditions, therefore, the binucleated condition would usually be of rather short duration. However, in maize-meal-agar plate cultures that are protected from rapid evaporation by being tightly covered with a bell jar, the binucleated condition is by no means ephemeral, but has been found to persist for 5 or 6 months, in many instances eventually even preponderating over the uninucleated state.

Several days after being planted such cultures are permeated so thoroughly by the fungus that virtually all further vegetative growth is precluded. Thenceforth the conidia, unable to produce mycelia, are limited mainly to reproductive development. At first the globose conidia (FIG. 1, *D–F; a; H, a–i*), all of them uninucleated like the hyphal segments from which they originated, nearly always give rise individually to a propulsive conidiophore that eventually shoots off a secondary globose conidium containing the single nucleus of its parent. Later this strictly repetitional development is supplanted in gradually increasing measure by the production of elongated adhesive conidia singly on solitary slender conidiophores. As in *Basidiobolus meristosporus* and *B. haptosporus* these slender conidiophores are not phototropic and apparently are never sent up from a hyphal segment. They are in many instances first sent up from scattered globose conidia (FIG. 3, *A, B*) when the Petri plate culture is 10 to 15 days old and has become overgrown to some extent by alien molds. Each elongated conidium (FIG. 3, *C, a–p*) produced by a uninucleated globose conidium receives the single nucleus of its parent. Although on a fresh substratum a uninucleated adhesive conidium may put forth a broad hypha (FIG. 3, *D*) capable of growing into a mycelium, in aging Petri plate cultures it more usually gives rise to a slender conidiophore on which another uninucleated adhesive conidium is produced. Successive generations of globose and of elongated conidia thus become intermingled everywhere

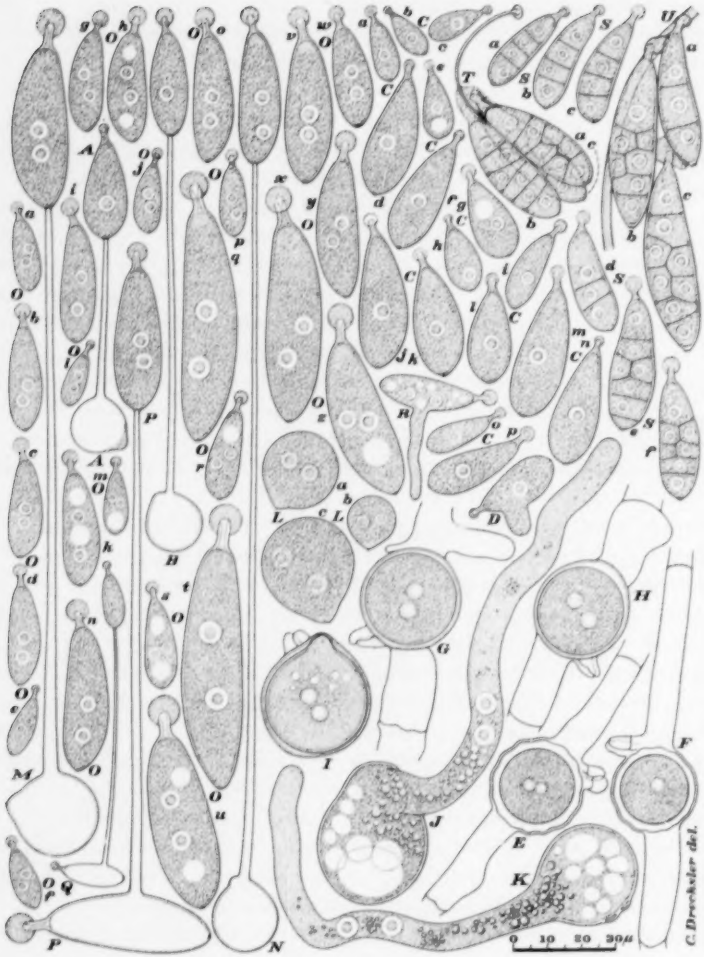

FIG. 3, *A–U*.

FIG. 3. *Basidiobolus ranarum* as found in maize-meal agar cultures, × 500. *A, B.* Globose conidia, each bearing a uninucleated adhesive conidium. *C.* Detached uninucleated adhesive conidia, *a–p*. *D.* Uninucleated adhesive conidium with broad germ tube. *E, F.* Mature zygospores showing no separation of wall into layers. *G, H.* Zygospores ready to germinate. *I.* Zygospore beginning to germinate. *J, K.* Zygospores, each with germ hypha showing 2 nuclei. *L.* Globose conidia, *a–c*, each with 2 nuclei. *M, N.* Globose conidia that have each produced

on the cultures, the uninucleated living specimens being found scattered among the empty membranous envelopes of their ancestors.

After being held for 30 to 40 days at temperatures near 20° C, some maize-meal-agar plate cultures not seriously overgrown with species of *Penicillium* or other strongly antagonistic molds will usually show germinating zygospores here and there. During an ensuing period of 10 to 20 days many other zygospores may likewise germinate, so that in cultures 50 to 60 days old most of the zygospores present originally may be represented only by their empty envelopes. Vegetative development being precluded, the broad germ tube extended in each instance grows out into a phototropic conidiophore which later shoots off a globose binucleated conidium (FIG. 3, *L*, *a–c*). This conidium, as might be expected, may give rise to a phototropic conidiophore that eventually discharges a secondary globose conidium containing 2 nuclei; or it may send up a slender erect conidiophore bearing aloft an elongated binucleated conidium with an adhesive beak (FIG. 3, *M*, *N*). Binucleated adhesive conidia thus formed, much like uninucleated adhesive conidia, become detached (FIG. 1, *Q–W*; FIG. 3, *O*, *a–z*) on relatively slight disturbance. In aging Petri plate cultures they commonly give rise individually to a slender conidiophore on which another binucleated adhesive conidium is produced (FIG. 3, *P*, *Q*), though they are capable of germinating vegetatively (FIG. 3, *R*) on a fresh substratum. Through continued repetitional development successive generations of binucleated conidia of both the globose and the elongated type are produced among globose and elongated uninucleated conidia derived earlier from hyphal segments. The distinction between uninucleated (FIG. 3, *C*, *a–c*) and binucleated (FIG. 3, *O*, *e*, *f*, *l*, *m*) individuals persists recognizably among the dwarfish adhesive conidia found in Petri plate cultures 5 to 6 months old.

While the adhesive conidial state of the odorous *Basidiobolus* appears remarkable in persisting through many generations despite the presence of alien microrganisms, it occurs also in pure cultures. In tube cultures of maize-meal-agar adhesive conidia are formed only rarely and sparingly on the slanted surface of the substratum, but are produced somewhat more often and in greater numbers on the glass

a binucleated adhesive conidium. *O*. Detached binucleated adhesive conidia, *a–z*. *P*, *Q*. Adhesive conidia that have each produced a binucleated adhesive conidium. *R*. Binucleated adhesive conidium germinating. *S*. Detached adhesive conidia, *a–f*, largely or wholly converted into sporangia. *T*, *U*. Groups of 3 adhesive conidia, *a–c*, fastened to bristle of a mite, the several conidia being largely or wholly converted into sporangia.

surface opposite the substratum. On the glass surface, besides, they more usually are converted into sporangia. In undergoing such conversion the smaller adhesive conidia become divided by several transverse walls (FIG. 3, *S, a–d*), whereas the larger ones become divided not only by transverse but also by longitudinal walls (FIG. 3, *S, e, f*). The resulting segments, or sporangiospores, always contain a single readily visible nucleus. After their release from the sporangial envelope they usually become more rounded. Many that acquire a nearly globose shape will then measure approximately 10 μ in diameter.

On the whole, sporangial segmentation in the odorous *Basidiobolus* takes place less abundantly and less regularly than in *B. meristosporus*. In Petri plate cultures infested with mites the frequently numerous elongated conidia adhering firmly to the hairs or bristles (FIG. 3, *T, a–c; U, a–c*) of the animals usually are found either converted into sporangia or in process of undergoing segmentation, though the conidia not affixed to mites may show segmentation in only a few instances. It can hardly be doubted that the burdened animals stimulate sporangial development. A chemical stimulus might be received more especially by conidia which are attached directly to a hair or bristle (FIG. 3, *T, a–c; U, a, b*). As segmentation takes place also in conidia (FIG. 3, *U, c*) that adhere to affixed conidia and consequently are not in direct contact with the mite, it seems not impossible that the gentle mechanical disturbance resulting from the locomotion of the burdened animal may have some influence on sporangial development.

If nutrients are wholly lacking, a secondary globose conidium of *Basidiobolus ranarum*, according to Eidam (**7**: 218, lines 4–13), occasionally gives rise on an extraordinarily slender conidiophore to a distal enlargement that after receiving the entire mass of protoplasmic materials puts forth at its apex a small tertiary conidium which may stop developing early in an immature unfinished state. Despite the puzzling implication conveyed in Eidam's text that the tertiary conidium, when not arrested in its development, would normally receive all the protoplasmic contents of the enlargement borne distally on the slender conidiophore, his revelant illustration (**7**: Pl. 9, Fig. 16) unquestionably represents a globose conidium that has given rise on a slender conidiophore to an elongated conidium with an adhesive beak. From the magnification indicated for the illustration the elongated conidium measures about 47 μ in length and 15 μ in greatest width. These measurements, which differ little from the measurements of the 2 conidia shown in FIG. 3, *C, j, m*, are approximately median in the dimensional ranges of the adhesive conidia produced by the odorous *Basidiobolus*—18 to 83 μ for length and 6 to 22 μ for greatest width.

The mites infesting old Petri plate cultures of the odorous *Basidiobolus* usually have numerous adhesive sporangia and conidia attached to their bristles, some of the animals being found laden with more than a hundred of the easily recognizable reproductive bodies. Only occasionally is a globose conidium found attached to a mite, the infrequent instances of such attachment resulting from accidental contact of the globose conidium with the adhesive material at the tip of an affixed elongated conidium. It may be presumed that under natural conditions the mites, spiders, and insects which habitually infest decaying plant materials likewise become somewhat abundantly contaminated with adhesive conidia and sporangia. Infection of the frogs that devour contaminated arthropods would seem therefore to come about very largely from the elongated reproductive bodies, and only in small measure from globose conidia. There is good reason to believe that if Eidam had known the true nature of the distended bodies he found borne aloft on slender conidiophores he would not have been so consistently unsuccessful in recognizing *B. ranarum* in the varied contents taken freshly from the stomach and intestines of frogs, and would have been able to explain much better how the fungus gains entrance into a frog's digestive organs.

The odorous *Basidiobolus*, unlike *B. meristosporus* and *B. haptosporus*, agrees well with *B. ranarum* in the undulated sculpture of its zygospores. It also agrees rather satisfactorily with the original characterization of *B. ranarum* in all other important aspects of morphology. In view of its abundant development in Maryland and Virginia before the onset of hot mid-summer weather, it would seem very well adapted to the cooler summer climate of central Europe where Eidam carried on his investigations. The odorous *Basidiobolus* occurring widely in the United States is accordingly held to be identical with *B. ranarum*.

GROWTH CHARACTERISTICS AND SEXUAL REPRODUCTION OF BASIDIOBOLUS HAPTOSPORUS

The original account (2) wherein *Basidiobolus haptosporus* is presented as a new species was based on an assortment of globose conidia, slender conidiophores, and elongated adhesive conidia found in a maize-meal-agar plate culture that after being planted with leaf mold in February, 1946, was kept tightly covered under a battery jar at temperatures between 18° and 20° C. From the small size of the globose conidia—the empty envelopes of these bodies (2: Figs. 1–4: a) commonly measured less than 25 μ in diameter—and the relatively low temperatures at which they developed, the assortment of asexual reproductive

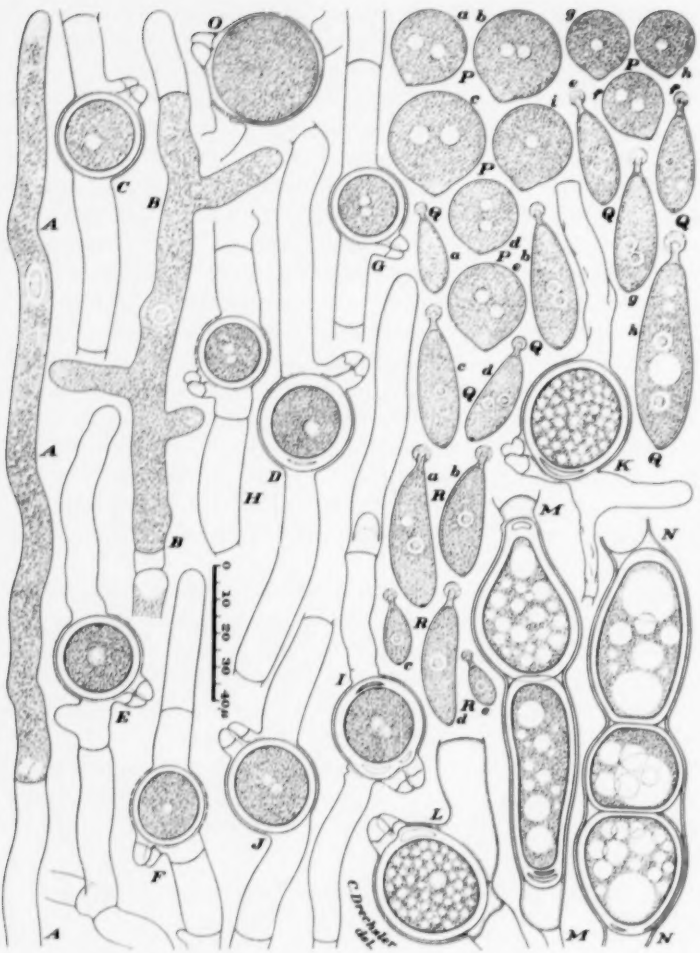

FIG. 4, *A–R*.

FIG. 4. *Basidiobolus haptosporus* (Lubber Run Park isolation) as found in maize-meal agar cultures, × 500. *A*. Terminal portion of hypha at margin of a growing mycelium. *B*. Terminal portion of hypha at margin of a mycelium not actively expanding. *C–J*. Mature zygospores produced in a Petri plate culture. *K, L*. Larger zygospores produced at a depth of 3 mm in a tube culture. *M, N*. Chlamydospores produced in a tube culture at a depth of 5 mm. *O*. Zygospore ready to germinate. *P*. Globose conidia among which six, *a–f*, show 2 nuclei, and

apparatus evidently belonged to the same species as the beaked smooth zygospores I have observed from time to time in maize-meal-agar cultures prepared for the isolation of oomycetous parasites from decaying roots (**6**). The fungus was first obtained in pure culture by canopying Petri plates of maize-meal-agar with leaf mold gathered in moist deciduous woods near Beltsville, Maryland, in December, 1951 (**3**, **4**). More than a score of additional isolations have since been obtained from isolation plate cultures canopied with leaf mold or other plant detritus collected in different localities on different dates, as follows: near Fort Myer in Arlington, Virginia, on January 22, 1952; in Lubber Run Park in Arlington, Virginia, on February 28, 1952; near Criglersville, Virginia, on March 23, 1952; near Marriotsville, Maryland, on March 18, 1952; near Middletown, Delaware, on February 27, 1953; and near Gumboro, Delaware, on March 7, 1953.

A mycelium of *Basidiobolus haptosporus* growing actively at temperatures between 18° and 20° C is often hardly visible to the naked eye. On careful scrutiny, however, a somewhat opaque circular band can be distinguished at its periphery. As the mycelium enlarges the circular band expands. The widening central area presents much the same appearance as the unoccupied substratum outside the band. After the band reaches the rim of the Petri dish it vanishes progressively, with the result that soon the culture again looks like a newly poured agar plate. In strong contrast to *B. meristosporus* the fungus does not usually form aerial hyphae in visible quantity. Development of subsidiary mycelia in the expanse of substratum extending toward the main source of light has not been observed often in cultures of *B. haptosporus*.

When the peripheral band of an actively growing mycelium is examined under a microscope, it is found to consist of the vegetative distal portions of radially arranged hyphae. The terminal segments of these hyphae (FIG. 4, *A*) often exceed 200 μ in length, and commonly vary from 8 to 11 μ in thickness. As they elongate in advancing the mycelial forefront they divide repeatedly, thereby cutting off one segment after another. If its elongation is obstructed a terminal segment (FIG. 4, *B*) may like many intercalary segments put forth one or more lateral branches. In a mycelium that has originated from a germinating conidium, the hyphal segments near the empty conidial envelope may measure 15 to 20 μ in width.

Along the inner margin of the visible band at the periphery of a

three, *g–i*, show 1 nucleus. *Q*. Adhesive conidia, *a–h*, each containing 2 nuclei. *R*. Adhesive conidia, *a–e*, each containing a single nucleus.

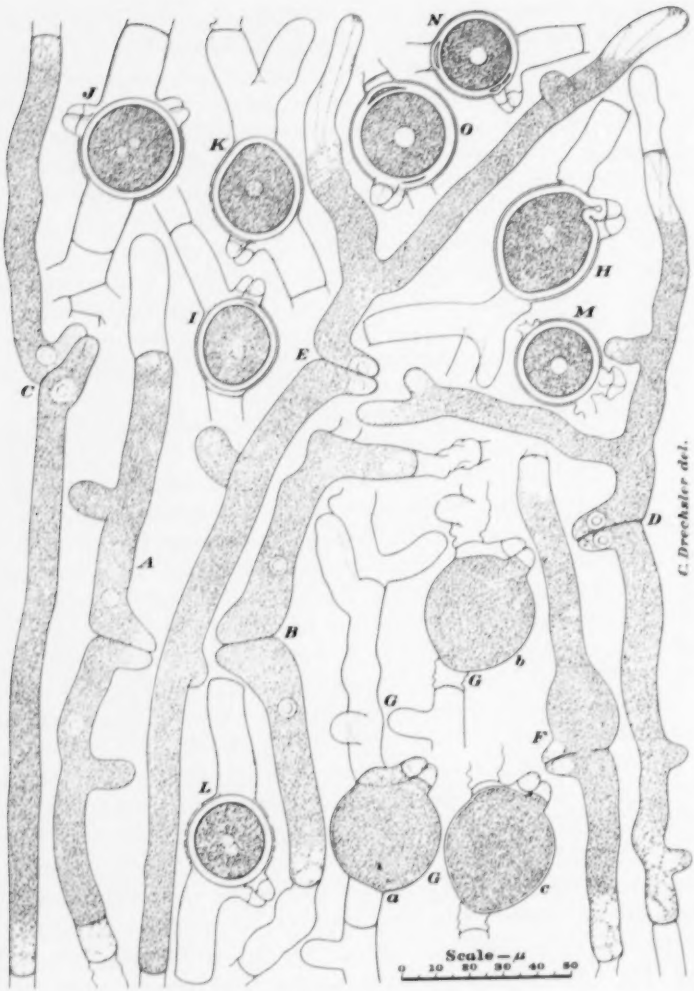

FIG. 5, *A–O*.

FIG. 5. *Basidiobolus haptosporus* (Lubber Run Park isolation) as found in maize-meal agar cultures, × 500. *A–E*. Young units of sexual reproductive apparatus; in *A* and *B* the 2 nuclei are moving toward paired protuberances, in *C* they have reached protuberances, in *D* they have entered protuberances, in *E* they have reached the tips. *F*. Sexual unit showing enlargement in which zygospore will develop. *G*. Sexual unit showing final stages, *a–c*, in conjugation. *H–O*. Zygo-

growing mycelium, paired hyphal segments are found in various stages of conjugation. Much as in other members of the genus, the paired segments extend juxtaposed protuberances (FIG. 5, *A, B*) into which the nuclei migrate (FIG. 5, *C–F*) to undergo division; the tip of each protuberance, partly filled with degenerating nuclear materials, being subsequently walled off. An aperture having meanwhile been formed in the cross-wall between the two segments, the protoplasm and nuclei of both segments collect in an enlargement (FIG. 5, *G, a–c*) on one side of the perforated partition. The enlargement, or fusion cell, now gradually undergoes conversion into a zygospore. At maturity the zygospore (FIG. 4, *C–L;* FIG. 5, *H–O*) is surrounded by a thick smooth wall which in most instances appears closely adnate to the thin hyphal membrane, so that the thin membrane can be distinguished clearly only where it would seem to have become fissured (FIG. 4, *E, F, H;* FIG. 5, *K, L*) or wrinkled (FIG. 5, *J*). The zygospore wall proper is rarely found separated throughout into 2 layers. It commonly either is solid throughout or shows localized separation into 2 layers in relatively small regions at the poles or under the protuberances (FIG. 4, *D, I, K, L;* FIG. 5, *I, N, O*). Some mature zygospores contain protoplasm of coarsely granular texture (FIG. 4, *C–J;* FIG. 5, *H–O*), while others seem filled with strongly globuliferous cytoplasm (FIG. 4, *K, L*). In many zygospores (FIG. 4, *C–F, K, L;* FIG. 5, *K, M, N, O*) a single nucleus can be made out clearly, whereas in others (FIG. 4, *G–J;* FIG. 5, *H–J, L*) two nuclei loom indistinctly through the dense protoplasm.

Sexual reproduction in *Basidiobolus haptosporus* appears to proceed less freely at depths of 2 to 3 mm than near the surface of the substratum. At depths of 4 to 5 mm in slanted tubes of maize-meal agar, zygospore formation is often completely inhibited, the submerged hyphal segments then usually becoming markedly distended and indurated. In many instances the swollen segments appear to contain two or more thick-walled endogenous chlamydospores (FIG. 4, *M, N*). The variously indurated cells seem to resemble rather closely the swollen thick-walled terminal cells that Eidam (**7**: 239, Pl. 12, Fig. 24) observed in preparations of his *B. lacertae*. They invite comparison, besides, with the thick-walled cells Raciborski (**14**: 114) obtained in glycerine cultures of *B. ranarum*, as well as with the resting stage (Dauerzustände) which Levisohn (**11**: 523) observed in cultures of *B. ranarum* amply supplied with air and contaminated with bacteria and molds.

spores and adjoining empty membranous parts, showing irregular zygospore wall in *H*, minute wrinkling or fissuring of the zygosporangial envelope in *J–L*, and localized separation of zygospore wall into 2 layers in *I, N, O*.

If maize-meal agar plate cultures of *Basidiobolus haptosporus* are protected against excessive evaporation by being covered tightly with a battery jar, many zygospores (FIG. 4, *O*) may be found after 50 to 60 days to have largely resorbed the thick wall present during the resting period. Where contamination by antagonistic molds is not too serious these after-ripened zygospores will germinate freely without irrigation or removal from the staled substratum, each putting forth a germ hypha terminating in a phototropic conidiophore from which eventually a globose conidium is shot off. As far as could be determined from unstained living specimens all globose conidia thus produced contain 2 nuclei (FIG. 4, *P, a–f*). Two nuclei are always discernible in elongated adhesive conidia (FIG. 4, *Q, a–h*) produced on slender conidiophores extended singly from globose conidia of zygosporic origin. Here, as also in *B. meristosporus* and *B. ranarum*, the binucleated condition will persist through many successive generations of repetitional development, being terminated, however, when the conidium becomes transformed into a sporangium through internal segmentation, or undergoes a single division preparatory to the production of a zygospore.

In *Basidiobolus haptosporus* conidia are produced far more abundantly from germinating zygospores than from hyphal segments. Often not a single broad conidiophore can be discovered when an extensive and actively growing mycelium is thoroughly explored under a microscope. Apparently in many Petri plate cultures of *B. haptosporus* asexual reproduction is wholly absent during the period of vegetative growth, all hyphal segments contributing their contents to the formation of zygospores. Yet now and then a small number of detached globose conidia are found scattered about in Petri plate cultures only a few days old. As far as could be determined from examination of unstained living specimens, the globose conidia in such young cultures always contain a single nucleus (FIG. 4, *P, g–i*). Only a single nucleus can be distinguished in the few elongated adhesive conidia (FIG. 4, *R, a–e*) sometimes found in cultures less than 20 days old. Since after-ripened zygospores have never been observed in cultures so young, these adhesive conidia must derive from globose conidia of mycelial origin.

The production of adhesive conidia can obviously no longer be held to distinguish *Basidiobolus haptosporus* as a separate species, as such conidia are now known to be formed also in *B. meristosporus* and *B. ranarum*. Nor can a diagnostic difference be recognized any longer in the difference between the strongly tapering shape of the terminal enlargement figured by Eidam (**7**: Pl. 9, Fig. 16) and the gently tapering

shape of the adhesive conidia present in the material on which my original description of *B. haptosporus* was based. In *B. haptosporus* and *B. meristosporus*, no less than in *B. ranarum*, a strongly tapering shape is usual among adhesive conidia formed on the glass surface opposite the slanted substratum in tube cultures, and would seem to result from dry conditions. Furthermore, in maize-meal-agar cultures of all 3 species a gently tapering shape is usual among adhesive conidia produced by parent conidia lying directly on the moist substratum, and must consequently be held to result from moist conditions. Presumably owing to lack of water the strongly tapering conidia formed on a glass surface by any of the 3 species are very sparingly tipped with sticky material, whereas gently tapering conidia of all 3 species commonly bear a massive globule of yellow adhesive substance at the apex. In *B. haptosporus* the adhesive conidia, as also the globose conidia and zygospores, do not attain maximum dimensions quite as large as in the generally more robust *B. ranarum*. They seem approximately equal in size to the adhesive conidia of *B. meristosporus*, but apparently are less prone to become segmented into sporangia.

Basidiobolus haptosporus is adequately distinguished from *B. ranarum* by its production of smooth rather than undulated zygospores, by its very meager production of conidia from its hyphal segments, and by its lack of any *Streptomyces*-like odor. It is clearly separated from *B. meristosporus* by its adaptation to lower temperatures, by its meager production of conidia from hyphal segments, and by its failure usually to produce aerial mycelium. It differs from the description of *B. myxophilus* R. E. Fries (**9**) in its smooth zygospores, and from the description of *B. lacertae* in its well-developed paired protuberances, which normally show a median septum. A revised diagnosis incorporating information on its vegetative development and its sexual reproduction may prove helpful in construing the species correctly.

BASIDIOBOLUS HAPTOSPORUS Drechsl. emend. Drechsl.

Mycelium inconspicuum, vulgo non in aerem visibiliter crescens, incoloratum; hyphis sterilibus ramosis, 3–20 μ (plerumque 8–11 μ) crassis, mox septatis, hic illic disjunctis, cellulis eorum plerumque 35–250 μ longis, uno nucleo visibili praeditis. Primiformibus fertilibus hyphis singulatim raro ex cellulis mycelii sed saepe ex conidiis vel ex zygosporis surgentibus, incoloratis, simplicibus, basi interdum 3.5–6 μ latis, in aerem vulgo 75–175 μ ad lucem protendentibus, sursum in tumorem jaculatorium aliquando 30–40 μ longum, 15–20 μ latum inflatis, apice unum primiforme conidium ferentibus, denique hoc violenter adjicentibus; primiformibus conidiis globosis sed basi ad instar mammiculae prominulis, plerumque 16–30 μ in diametro. Hyphis formae gracilis fertilibus ex primiformibus vel tenacibus conidiis

nec umquam ex cellulis mycelii surgentibus, incoloratis, rectis, 50–325 μ longis, basi 1.5–4.5 μ crassis, sursum leniter attenuatis, apice 1–2 μ latis, ibi unum conidium tenax ferentibus. Tenacibus conidiis in totum 17–73 μ longis, ex infera viventi cellula et supero glutinoso rostro constantibus; glutinoso rostro flavido, tubulato, 3–8 μ longo, sursum 1–2.5 μ lato, apice vulgo guttula materiae glutinosae flavae 2–10 μ crassa vestito; viventi cellula incolorata, elongato-ellipsoidea, recta vel leviter curvata, 13–61 μ longis, 6–18 μ latis, uno nucleo vel duobus nucleis instructis, quandoque in sporangium transeunte; sporis uno nucleo praeditis, incoloratis, primo disciformibus vel dolioformibus, postea plus minusve rotundatis et saepe circa 10 μ in diametro. Zygosporis ex conjugio duarum cellularum contiguarum oriundis, globosis vel elongato-ellipsoideis, plerumque 23–37 μ longis, 21–34 μ latis, muro levi saepe aliquid flavido 2–3.5 μ crasso circumdatis.

Habitat in materiis plantarum putrescentibus in Virginia, Maryland, Delaware, Wisconsin.

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THE CYTOLOGY OF SYZYGOSPORA ALBA [1]

C. J. KAO [2, 3]

(WITH 28 FIGURES)

The genus *Syzygospora* Martin (3) was based on collections made in 1935 in western Panama in the mountain forest of Chiriqui. Macroscopically, the fungus is a large (10–12 cm or more in diameter), white, soft-gelatinous species looking like a *Tremella* and growing on oak logs and stumps. Microscopically, it proved to have a hymenial surface composed of 2-celled, basidium-like, spore-bearing structures, each of which appeared to bear two sessile, globose spores, one from each cell, which fused before discharge. On the basis of the septate, basidium-like structure, Martin referred the genus to the Auriculariaceae. He noted occasional basidia that appeared to bear two spores from each cell.

In 1952, additional collections were made by Martin and Welden at Casita Alta above Boquete, on the opposite side of the Chiriqui volcano from which the earlier collections had been made.

MATERIALS AND METHODS

The material used in this cytological study was that collected by Martin and Welden in August, 1952. The specimen (GWM *8081*) was fixed in FPA solution in the field and kept in the solution until October. The material was then dehydrated in a butyl alcohol series and was imbedded in paraffin and sectioned at 6 μ. Three staining methods were used for this study. Heidenhain's Iron-alum Hematoxylin staining technique (1) with Orange G or Fast Green as counterstain proved most successful in studying the nuclei of this fungus.

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the State University of Iowa, June, 1955.

[2] The writer wishes to express her gratitude to Professor G. W. Martin for his guidance and encouragement during the course of this study. She also wishes to acknowledge the Nancy Skinner Clark Fellowship for 1952–53 from Vassar College and the Frank M. Shu Scientific Fellowship for 1954–55 from the China Institute in America, without which this work could not have been done.

[3] Mrs. Stephen S. Wang, Jewish Hospital Clinical Laboratory, Cincinnati 29, Ohio.

Flemming's triple stain (1) and the Safranin and Fast Green (1) staining method also gave good results.

A dry specimen, which was soaked in water and later preserved in 5% formalin solution, was used for the morphological study. A small piece of fungus tissue stained with KOH-Phloxine was found useful. A very dilute Crystal Violet solution also gave satisfactory results in studying the structure of hyphae, basidia and basidiospores.

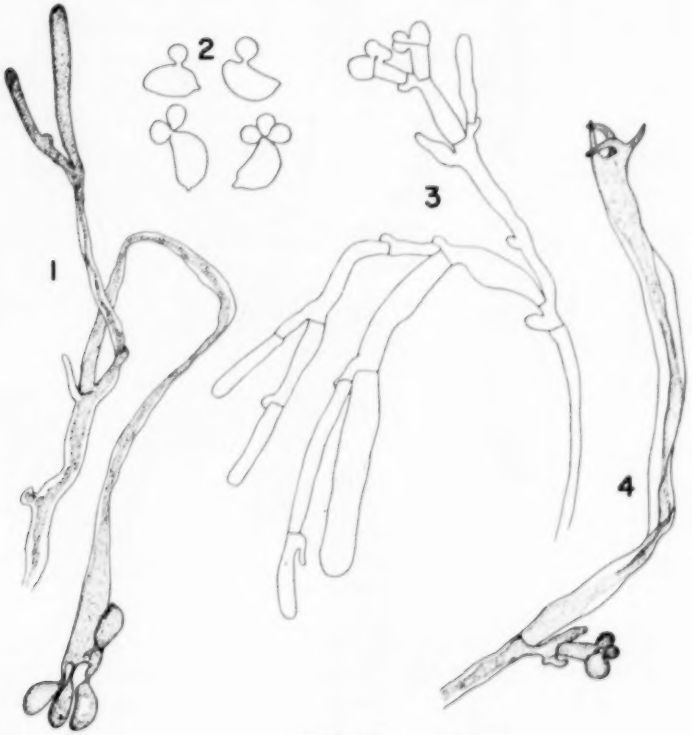
OBSERVATIONS AND RESULTS

The specimen in 5% formaline shows a sessile, dingy white and gelatinous basidiocarp. A small piece of the fungus tissue was stained with KOH-Phloxine or Crystal Violet and mounted under a cover glass. The cover glass was then pressed gently in order to separate the hyphae and basidia. The hyphae are usually interwozen. This hyphal formation gives rise to numerous radiating branches mostly terminating in what Martin (3) regarded as basidia. Clamp-connections are abundant. The "basidia" are borne, for the most part, in unilateral cymose tufts of two to four along the hyphae (FIG. 3) and are transversely septate into two cells. The terminal cell is blunt and approximately isodiametric. The basal cell is longer and attenuated toward the base. Each cell produces a globose spore, the basidiospore according to Martin, on a short stalk. Usually the globose spores from both the terminal and the basal cell are formed close to the septum. The spores fuse, after which the resultant, binucleate, dumbbell-shaped fusion spore is detached from the hyphal branch. Frequently two pairs of spores can be seen on one "basidium." The same hypha bears large, long hyphal cells which Martin called cystidia (FIG. 3).

In addition to the structures described above, some large homobasidiomycete-type basidia are present in this specimen (FIG. 1). The hyphae which bear the homobasidiomycete-type basidia cannot be differentiated from those hyphae which bear the *Syzygospora*-type "basidia" (FIGS. 1, 3). Furthermore, in one instance, one hypha was observed bearing both types of basidia (FIG. 4). This indicates that the *Syzygospora* is probably a conidial stage of the homobasidiomycete. However, only *Syzygospora* spores can be seen in the cotype specimen (GWM *2167*).

In the prepared slides, the hymenium may consist of numerous, regular, straight, dicaryon hyphae with homobasidiomycete-type basidia. As contrasted with this, some hymenial layers are mainly composed of irregularly branched, short, dicaryon hyphae bearing conidia, the *Syzy-*

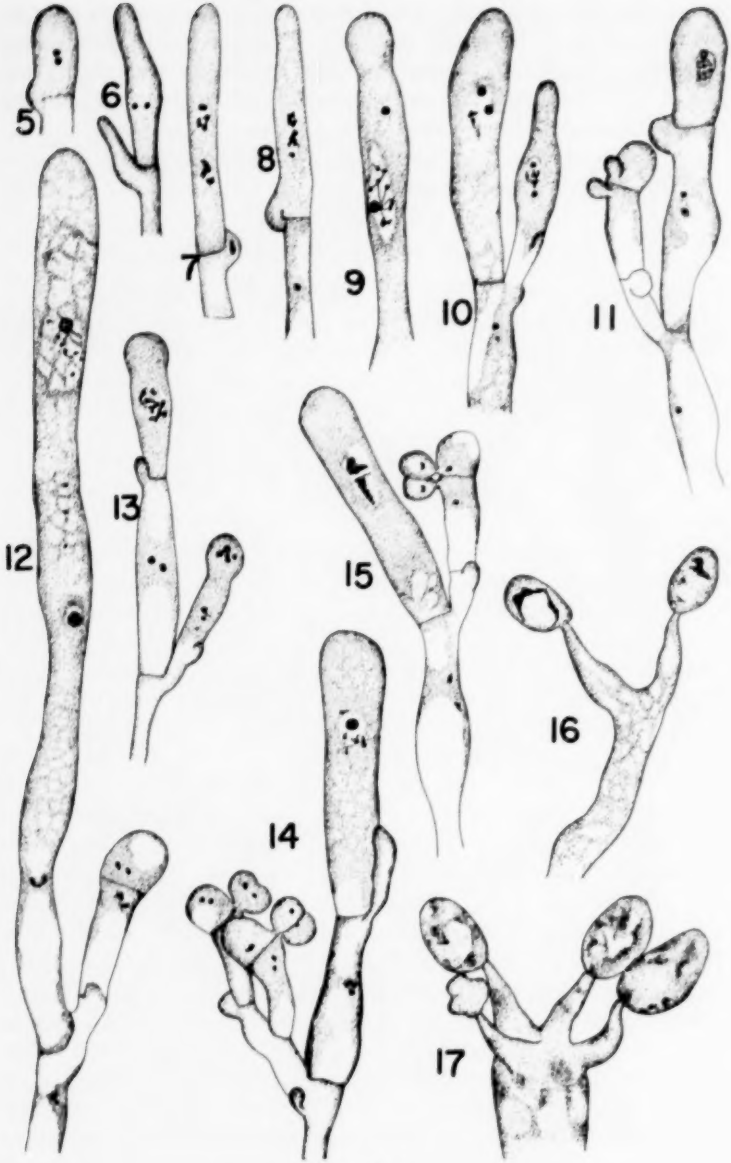
gospora "basidia" of Martin. In other instances, a mixture of the two types of hymenia is frequently observed. The nuclei are stained deep purple with Iron-Alum Hematoxylin; purplish red with the triple stain and red with Safranin. No nuclear membrane has been seen and the nuclear contents are not discernible.



FIGS. 1–4.

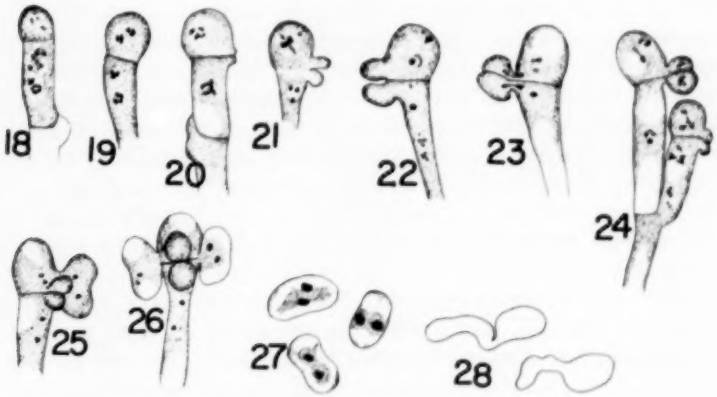
The terminal hyphae can be differentiated into three types: (1) long, slender (FIG. 7); (2) stout, with blunt tip (FIG. 5); (3) finger-shaped, with broad middle portion and tapering tip (FIG. 6). Fusion nuclei have been found in the terminal cells of all three types of hyphae (FIGS. 8, 10, 11). Branching usually originates at the clamp-connections, as shown in FIGS. 10, 11, 12, 13, 14, 15.

FIG. 10 illustrates the presence of young probasidia at the tips of the hyphae. These structures are characterized by a large diploid



FIGS. 5–17.

nucleus without membrane, the chromatin material being aggregated into a mass or scattered granules. The nuclei in FIG. 10 are probably in early prophase. FIGS. 11, 14 suggest the later stages of prophase. Only one nucleolus appears in these nuclei, which may be explained as a result either of fusion of nuceoli or of degeneration. FIG. 15 indicates a later stage of nuclear division. The relation between the stages of nuclear division and the growth in basidium size is not well established. In some instances the probasidia are small in size yet the nuclear division is in a similar stage to that which may be seen in later probasidia.



FIGS. 18–28.

Since only a very small number of the homobasidiomycete-type basidia are found in the fruiting body, the nuclear behavior in the basidium cannot be precisely followed. A typical basidium has four sterigmata from which four basidiospores are produced (FIG. 17). Sometimes, the basidium produces two (FIG. 16) or three sterigmata and basidiospores. Basidiospores are occasionally found germinating while still attached to the sterigmata. Basidiospores (6–11 $\mu \times 3.5$–5 μ) germinate by producing mycelium or by forming blastospore-like conidia (FIG. 2).

It is difficult to differentiate the hyphae which are destined to produce the *Syzygospora*-type of "basidia" from those which will produce the homobasidiomycete-type, and, as stated previously, both structures were, in one instance, observed on the same hypha, with other suggestions of the same thing, e.g. FIGS. 14, 15. It seems that any hyphal tip,

if no karyogamy occurs, will develop into the *Syzygospora* stage (FIG. 18). FIG. 19 shows the hyphal tip (7–12 × 3–4 μ) separated into two cells by a transverse septum. The nucleus in each cell divides to form two nuclei. The next stage is the formation of two protuberances near the cross wall (FIG. 20). One rises from the terminal cell and the other rises from the basal cell. They are approximately equal in size (FIGS. 21, 23). Two nuclei, one from the terminal cell, one from the basal cell, migrate into these structures and form two external spores (FIGS. 23, 24). These two spores usually grow side by side and fuse to form a dumbbell-shaped spore which then becomes detached(FIG. 25).

During the process of formation of the first pair of spores, the nucleus in the terminal cell may divide to form two nuclei (FIG. 21). More or less simultaneously the nucleus in the basal cell also divides into two nuclei. One protuberance rises from each cell and a second pair of spores is produced (FIG. 25). A hypha may often be seen bearing one fused, cylindrical, binucleate spore ready to be discharged and two young spores not yet fused. Two pairs of already fused spores growing on one hypha may also be observed frequently. After the second pair of spores is formed, there is still nuclear material in the cells. Occasionally three pairs of spores can be seen on one hyphal tip (FIG. 26).

The dumbbell-shaped spores (5–6 × 2.5–3 μ) are very abundant. They are dicaryon (FIG. 27). Sometimes the nuclear material is present in great amount. Frequently these two nuclei are very close to each other and in a few cases only one nucleus can be found in this fused spore. These spores germinate by producing a mycelial thread (FIG. 28).

DISCUSSION

The spore-bearing hyphae and the spores of *Syzygospora* as described by Martin (**3**) bear resemblance to the basidia and the basidiospores of *Stilbum vulgare* Tode (**2**), in spite of the fact that no fusion of spores occurs in the latter species. In FIG. 13, the shape of the hyphae suggests very much that they are giving rise to *Syzygospora* type "basidia." The fusion nucleus in one terminal hyphal cell is preparing to divided into two daughter nuclei; in the other, division has already occurred, and one daughter nucleus has migrated into the swollen and blunt terminal part of the hypha. A transverse septum will form between these two nuclei before the spores are produced. Thus, *Syzygospora* appears to be a member of the Heterobasidiomycetes. However, since *Syzygospora*-type "basidia" and the homobasidiomycete-

type basidia are found on the same hyphae, this introduces an element of uncertainty into the assumption that the probasidium shown in FIG. 13 will develop into *Syzygospora*. Besides, as shown in FIG. 9, the large homobasidiomycete hyphae also possess the blunt, isodiametrical outgrowth. Morphologically varied appearances of the probasidia are very common in this fungus.

Since the *Syzygospora*-type basidium and the homobasidiomycetous basidium are borne on the same hypha, it may be inferred that the *Syzygospora* is probably only a conidial stage of the homobasidiomycete. Taxonomically, it is impossible to identify this fungus because the fruiting body consists mainly of conidia and hyphae. No such kind of conidial form has been described in the literature.

Cystidium-like structures have been observed in this study which are characterized by more or less tapering hyphae, 3 μ in width, emerging about 10–25 μ above the hymenial layer, which is a stratum composed mainly of conidia with occasional probasidia. In the densely interwoven hyphae of the hymenial layers, cytoplasm may be observed in the hyphae but no nuclei have been seen. The cystidium-like structures are small as compared with typical cystidia. If they are so designated, then the finger-shaped hyphal cells with broad middle portion and tapering ends (FIG. 6) are probably cystidium initials in which the nuclear fusion occurs (FIG. 10) followed by division or degeneration as reported by Whelden (**5**) in *Peniophora livida* and by Ritchie (**4**) in *Russula emetica*. In this respect, the present study is in agreement with the findings of these authors.

SUMMARY

The application of Heidenhain's Hematoxylin and KOH-Phloxine staining technics for the cytological study of *Syzygospora alba* suggests the following conclusions:

1. *Syzygospora*-type "basidia" and the homobasidiomycete-basidia are borne on the same hypha; thus *Syzygospora* is only a conidial stage of a homobasidiomycete.

2. The nuclear behavior in the development of the *Peniophora*-type basidium could not be precisely followed. The mature basidium usually produces four, occasionally two or three, sterigmata which are tipped by basidiospores. Basidiospores germinate by forming mycelium or by producing blastospore-like conidia.

3. The nuclear behavior in the development of the *Syzygospora*-type spores has been observed. The terminal hyphal cell becomes trans-

versely septate. Each daughter cell produces a short stalk on which a spore is borne. Two spores, one from each cell, fuse to form a binucleate dumbbell-shaped spore which becomes detached after it reached maturity. Spores germinate by producing mycelium.

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SPONTANEOUS MUTATION IN GELASINOSPORA CALOSPORA, A HOMOTHALLIC FUNGUS [1, 2]

G. B. Wilson and Const. J. Alexopoulos [3]

(WITH 1 FIGURE)

Gelasinospora calospora (Mouton) Moreau & Moreau is a homothallic fungus which was isolated in France in 1949 from soil obtained from a palm plantation in the French Congo (**1**). A single spore isolate from a transfer sent to us by Prof. Claude Moreau in 1950 has been in culture in our laboratory since that time. Because our work here was concentrated chiefly on the heterothallic form of the organism (var. *autosteira*) (**3**), only the relatively few transfers of the homothallic fungus required to maintain the organism in stock culture have been made. All stock cultures have been kept continuously in a refrigerator at approximately 6° C.

OBSERVATIONS

In the winter of 1955, while attempting to cross *G. calospora* with its heterothallic variety *autosteira*, it was noted that the *G. calospora* control plates produced a number of perithecia which contained many segregating asci along with the normal ones. The segregating asci generally contained 4 black spores, and different combinations of white and brown spores. The fact that, with rare exceptions, the two members of each spore pair were alike, and the fact that the three colors: black, brown, and white were distributed in all possible combinations, indicated that we were dealing with a genetically controlled factor which segregated in Mendelian fashion.

Normally, the ascospores of *G. calospora* pass through the following color sequence as they mature: white, yellowish, light brown, green, greenish-black, "black." At first, therefore, we believed that we were

[1] Contribution No. 56-7 from the Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan.

[2] Published as excess pagination at the expense of the All-College Research Committee, Michigan State University.

[3] Present address: Department of Botany, State University of Iowa, Iowa City, Iowa.

dealing with a factor which delayed maturity and that, as the perithecia aged and the spores matured, the pattern of segregation would change from tricolor to bicolor to unicolor. This did not prove to be the case. Perithecia old enough to have expelled most of their spores, proved, upon

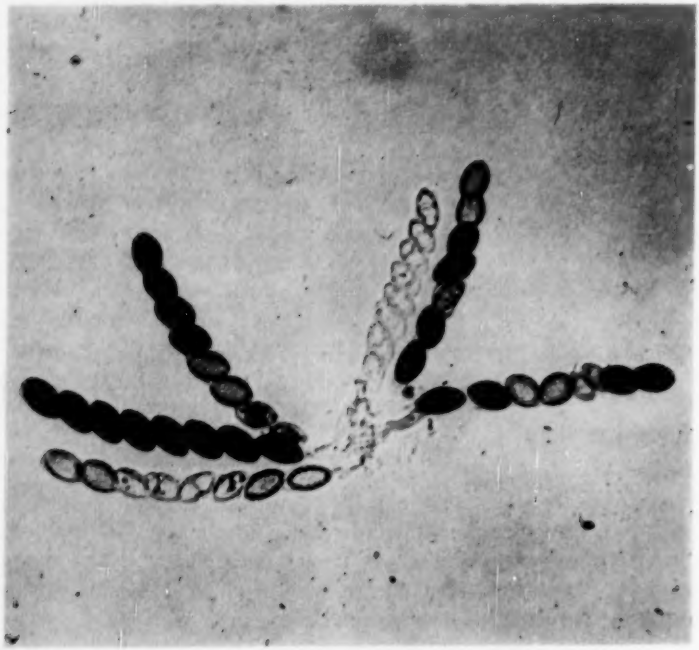

FIG. 1. Six asci of *Gelasinospora calospora* dissected out of an old perithecium which had discharged most of its spores. Color of spore pairs from top to bottom of each ascus, asci from left to right, as follows: Ascus 1, brown, white, white, brown; ascus 2, all black; ascus 3, black, black, brown, white; ascus 4, all white; ascus 5, brown, black, white, black; ascus 6, black, white, brown, black.

The two spores at the bottom of ascus 3 exhibit a peculiar condition which was found not infrequently during these observations. The spores, obviously immature, were filled with greenish particles, reminiscent of plastids, probably products of protoplasmic disintegration. This type of spore was classed as white.

dissection, to contain a number of tricolor asci as well as bicolor and unicolor (all white, all brown, or all black) asci, and in approximately the same ratio as younger mature perithecia (FIG. 1).

The segregation pattern was briefly as follows: The asci in the very young perithecia contained nothing but white spores, since all the spores,

regardless of genetic constitution, are hyaline at an early stage. In the next stage of development, the non-segregating asci contained all brown spores, whereas the segregating asci contained white and brown spores in various combinations. At maturity, the non-segregating asci contained 8 uniformly black spores, whereas the great majority of the segregating asci contained three types of spores: black, brown, and white, with generally two pairs of black spores. However, a relatively small number of asci contained 4 black and 4 white spores. No asci have ever been found with 4 black and 4 brown spores (TABLE I).

No evidence of preferential karyogamy, such as that reported by Olive (2) in *Sordaria fimicola* was noted; normal asci were always present along with the segregating ones in the same perithecium.

Mass mycelial transfers and a large number of hyphal tip isolations all yielded colonies which produced both normal perithecia and perithecia containing segregating along with normal asci, indicating a high degree of heterokaryosis in the mycelium.

Single black-spore isolates always yielded homokaryotic mycelium, as evidenced by the fact that no segregating asci were ever found in the perithecia which such isolates produced. To date we have not been able to germinate any of the brown or the white spores, either those we dissected from the asci or those which had been naturally expelled from the asci onto the agar medium.

DISCUSSION

Heterokaryosis. The observations recorded above show that there can be no doubt that the mycelium of the stock culture used in these experiments is heterokaryotic since: a) non-segregating and segregating perithecia are distributed at random in a single colony, b) all of the segregating perithecia contain some non-segregating asci in various proportions, and c) most important perhaps, transfers, either mass mycelial or hyphal tip, yield segregating colonies. In this last connection it might be pointed out that third or fourth transfer hyphal tips sometimes yield a smaller proportion of segregating perithecia, an expected result if the wild type nucleus is in excess of the mutant in a heterokaryotic mycelium. It should be theoretically possible to isolate homokaryotic wild type and mutant type mycelium by hyphal tip isolation if uninucleate tips, or tips with a small number of nuclei can be induced to grow.

Genetic interpretation of results. The segregation pattern in general, suggests the presence of two mutant genes, both of which delay maturity of spores, one of them (M_2) very slightly, the other (M_1) preventing maturity beyond the brown stage. Furthermore, when these mutants

are occurring together, they have a cumulative effect since the double mutant spore does not mature beyond the white stage.

With reference to the location of these mutants, a number of possibilities exist, such as: 1) having them in different nuclei, 2) in the same nucleus but on different chromosomes, or 3) linked in the same chromosome. An analysis of the results to date indicates some form of the last type of association, namely, linkage. This sets up the problem of choosing the relation of the two genes to each other and to the kinetochore. The only order which does not give us an expectation not so far

TABLE I

GELASINOSPORA CALOSPORA

ASCI EXPECTED IF MUTANT GENES ARE LINKED K – M_2 – M_1

Cross-over type	Ascal type		Phenotype	Observed	Expected
None	M_2 M_1 M_1 M_2	+ + + +	ww/BB	14	8
K – M_2	M_2 + M_1 +	+ M_1 + M_2	wB/Bw	7	8
M_2 – M_1	M_2 M_1 + M_2	+ M_1 + +	Bw/Bb	164*	176
2 Strand Double 4 Strand Double	M_2 + + +	M_1 M_2 + M_1	BB/bw	145	283
3 Strand Double	+ M_1 + M_2	M_2 + + M_1	Bw/Bb	145*	

* Calculated from total observed asci of type Bw/Bb.

Key to symbols: B = black; b = brown; w = white.

achieved is the K–M_2–M_1. On this order, no categories not observed are expected, whereas all categories observed are. Furthermore, in so far as valid numerical expectation can be set up, their fit is good (TABLE I).

This explanation indicates that two mutants affecting the same character have occurred perhaps simultaneously, on the same chromosome at different loci, in a relatively inactive culture, stored at low temperatures. Improbable as this explanation may sound, it nevertheless appears to be the one which explains most of the facts satisfactorily. Distribution of the mutant nuclei is, to a large extent, equivalent to that of the wild type, since all hyphal tip or mass mycelial transfers, regardless of the position on the culture from which they were taken, produce segregating asci.

SUMMARY

Two spontaneous mutations affecting spore maturation are reported in *Gelasinospora calospora*, a homothallic fungus. It is concluded that the mutations occurred in two loci on the same chromosome and that the mutant nuclei are widely distributed in the heterokaryotic mycelium.

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A NEW GENUS OF THE TREMELLACEAE [1]

MARION D. ERVIN [2]

The genus *Seismosarca* was established by Cooke (Grevillea **18**: 25. 1889) for a gelatinous fungus collected in New South Wales, which he called *S. hydrophora*. Little attention was paid to the genus until Lloyd (Myc. Writ. **5**, Letter 62: 6–7. 1916) referred a second collection from Australia to Cooke's species, noting its possession of gloeocystidia. Lloyd had examined Cooke's type at Kew and pointed out the errors in Cooke's description. The following year (Myc. Writ. **5**, Myc. Notes 629–630. 1917) he comments further on the second collection, stating that the spores are pale yellow and that the basidia are like those of an *Exidia*. Lloyd had previously shown that a common species of temperate North America, often listed as *Tremella albida* Huds. ex Fries, the identity of which is not certain, was unlike any European species and proposed for it the name *Exidiopsis alba* (Myc. Writ. **4**, Letter 44: 8–9. 1913). In 1917, he decided that *Seismosarca hydrophora* and *Exidiopsis alba* were congeneric and transferred the latter to *Seismosarca* as *S. alba* (Lloyd) Lloyd. Burt (Ann. Missouri Bot. Gard. **8**: 366. 1921) transferred the American species to *Exidia* as *E. alba* (Lloyd) Burt, noting that it would be the only member of the genus with gloeocystidia, but adding the comment: "it seems unnecessary and a great pity to segregate already small genera on the basis of every positive character which would make a species noteworthy."

Another species which must be considered in this connection is *Tremella pululahuana* Pat. (Bull. Soc. Myc. Fr. **9**: 138. 1893), originally described from Ecuador and also characterized by gloeocystidia. Bourdot and Galzin (Hymen. Fr. 48. 1929) transferred it to *Bourdotia*, believing it to be represented in Europe by two subspecies, *B. pululahuana* ssp. *Galzinii* (Bres.) B. & G. and *B. pululahuana* ssp. *caesia* (Bres. & Torr.) B. & G. Rogers (Univ. Iowa Stud. Nat. Hist. **17**: 38. 1935) transferred Patouillard's species to *Sebacina* as *S. pulu-*

[1] Based on a thesis submitted to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Doctor of Philosophy, under the direction of Professor G. W. Martin.

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lahuana (Pat.) Rogers, embracing under this name the subspecies recognized by Bourdot and Galzin and various other species which had been referred to *Sebacina* and *Bourdotia*, regarding *Bourdotia* as a subgenus of *Sebacina*. McGuire (Lloydia **4**: 33. 1941) treated *Bourdotia* as a section of *Sebacina* and referred the temperate collections to *S. Galzinii* Bres., including in that species *B. caesia* Bres. & Torr., *B. pululahuana* in the sense of Bourdot and Galzin and *S. pululahuana* in the sense of Rogers as synonyms, but excluding *Tremella pululahuana* of Patouillard.

Martin, in his earlier paper on the North Central Tremellales (Univ. Iowa Stud. Nat. Hist. **18**(3): 60–61. 1944), accepted the genus *Seismosarca* in Lloyd's sense, recognizing two species, *S. hydrophora* Cooke to include the yellowish, mainly tropical collections, and *S. alba* Lloyd for the white temperate species. However, after examining the type of *S. hydrophora* at Kew, Martin (Mycologia **43**: 112–113. 1951) concluded that it was an *Auricularia*. He therefore suggested that *S. alba* Lloyd be retained in *Exidia*, where Burt had placed it, and the tropical species be restored to *Tremella*, to which Patouillard had originally assigned it. In the revision of the North Central Tremellales (Univ. Iowa Stud. Nat. Hist. **19**(3): 78. 1952), Martin suggested that *E. alba* might "have to be referred to another, as yet unnamed, genus.

Microtome sections of the following species of *Exidia* have been studied: *E. glandulosa* Fries, *E. nucleata* (Schw.) Burt, *E. recisa* Fries, *E. saccharina* Fries, *E. cartilaginea* Lund. & Neuh., *E. villosa* Neuh. and *E. alba* (Lloyd) Burt. As compared with the other species, *E. alba* is much more stratose in section and lacks the tough epihymenial layer characteristic of *Exidia*. The only *Exidia* characters of *E. alba* are the allantoid spores and the pileate fructification. Spore shape is scarcely a good generic character in this group, and the fructification, although erumpent and pileate, is subfleshy. The species is not at home in *Exidia*, it does not fit in *Tremella* and it is quite different from *Bourdotia*, the only genus characterized by possession of gloeocystidia, in the nature of the basidiocarp, in its internal morphology and in the arrangement of the basidia.

Tremella pululahuana Pat., in microtome section, has the same stratification as *E. alba*, and similar pileate habit, gloeocystidia and basidial arrangement and development. It appears to be more gelatinous, is ochraceous rather than white or pinkish and the spores are yellowish in mass. I agree with Lloyd that the two species are congeneric.

Since the genus *Seismosarca*, to which both species were assigned by Lloyd, is probably based on an *Auricularia*, I am proposing a new genus to accommodate these and related species.

Gloeotromera gen. nov.

Fungi heterobasidiales, pileati, gelatinosi, ceraceo-gelatinosi vel subcarnosi, undique gloeocystidiati; probasidia subglobosa vel ovata, demum cruciato-septata; gloeocystidia subcylindracea, flexuosa, demum flava; sporae continuae, hyalinae vel flavescentes, per repititionem germinantes.

Etym. γλοία + τρομέω.

Fructification pileate, gelatinous, waxy-gelatinous or subfleshy, cerebriform or coarsely convolute; hymenium usually inferior or lateral; probasidia subglobose or oval, becoming cruciate-septate; gloeocystidia present, subcylindric, flexuous, originating below basidia, hyaline or granular, at length yellowish; spores hyaline, white or pale yellowish in mass, simple, germinating by repetition.

TYPE: *Exidiopsis alba* Lloyd.

The two species studied are transferred to the new genus and may be separated on the basis of the following key:

Waxy-gelatinous or subfleshy; white or pinkish to pale ochraceous; spores white in mass. Known only from temperate North America.....*G. alba*
Gelatinous; ochraceous to ochraceous-brown; spores pale yellowish in mass. Widely distributed, mainly in the tropics of both hemispheres...*G. pululahuana*

Gloeotromera alba (Lloyd) comb. nov.

Exidiopsis alba Lloyd, Myc. Writ. **4**, Letter 44: 8. 1913.
Seismosarca alba (Lloyd) Lloyd, Myc. Writ. **5**, Myc. Notes 45: 629. 1917.
Exidia alba (Lloyd) Burt, Ann. Missouri Bot. Gard. **8**: 366. 1921.

Subfleshy to waxy-gelatinous, cerebriform or coarsely convolute, white or pinkish to pale ochraceous, drying brown to blackish; probasidia subglobose or oval, about $10 \times 9\,\mu$, becoming longitudinally cruciate-septate; gloeocystidia subcylindrical, flexuous, originating below basidia, filled with white granular contents, becoming tardily yellowish, up to $30 \times 6\,\mu$; spores allantoid, hyaline, white in mass $8–11 \times 4–5\,\mu$, germinating by repetition.

Gloeotromera pululahuana (Pat.) comb. nov.

Tremella pululahuana Pat., Bull. Soc. Myc. Fr. **9**: 138. 1893.
Seismosarca hydrophora Cooke *sensu* Lloyd, Myc. Writ. **5**, Myc. Notes 45: 629. 1917. Not *Seismosarca hydrophora* Cooke, 1889.
Bourdotia pululahuana (Pat.) Bourd. & Galz., Hymen. Fr. 148. 1929.

Sebacina pululahuana (Pat.) Rogers, Univ. Iowa Stud. Nat. Hist. **17**: 38. 1935.

Fructification gelatinous, subpileate or effused, yellowish to ochraceous brown, cerebriform, probasidia subglobose to ovoid, 11–14 × 8–11 μ, becoming cruciate-septate; gloeocystidia subcylindric, flexuous, originating below hymenium and often extending through several growth layers, yellow from an early stage, 55–70 μ in length, spores hyaline, yellowish in mass, smooth, ovoid, curved, 10–12 × 5–6 μ, germinating by repetition.

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NORTH AMERICAN SPECIES OF THE GEOGLOSSACEAE. TRIBE CUDONIEAE[1]

E. B. Mains

(WITH 23 FIGURES)

The family Geoglossaceae was formerly treated as a subfamily of the Helvellaceae. In recent classifications where Boudier's separation of the Discomycetes has been followed the Geoglossaceae has been recognized as a family in the Inoperculates and therefore far removed from the Helvellaceae in the Operculates. In Nannfeldt's (**1932**) treatment of the Discomycetes it is placed in the Helotiales and is recognized as closely related to the Helotiaceae. As distinguished from the Helotiaceae, the Geoglossaceae is usually separated by having clavate, capitate or pileate ascocarps with the hymenium covering the convex upper portion and the Helotiaceae having discoid, saucer-shaped or cupulate ascocarps (Durand **1908**, Nannfeldt **1932**, Martin **1940**, Seaver **1951**).

In this study, Durand has been followed, with some modifications in the division of the Geoglossaceae into two tribes, the Geoglosseae and the Cudonieae. The Geoglosseae, with ascocarps that are capitate or clavate, has been treated in previous papers (Mains **1954**, **1955**). The Cudonieae, as discussed here, has ascocarps that are pileate. Imai (**1941**) has treated these taxa as subfamilies, Geoglossoideae and Cudonioideae, and has recognized a third subfamily, the Hemiglossoideae, for one genus *Hemiglossum*, which has branched coralloid ascocarps with unilateral hymenia. This genus has not been reported for North America.

The genera of the Cudonieae appear to occupy positions intermediate between the Geoglosseae and the Helotiaceae. *Leotia* has multiguttulate ascospores (FIG. 3) similar to those found in *Microglossum* of the Geoglosseae but the structure of its ascocarps differs from that of all of the other genera of the Geoglossaceae. The ascocarps consist of a central tissue of gelatinous hyphae separated from an outer layer of gelatinous hyphae by a distinct layer of non-gelatinous hyphae (FIGS. 2, 8). This is a structure which is found in *Ombrophila* and *Ascotre-*

[1] Paper from the Herbarium and the Department of Botany of the University of Michigan.

FIGS. 1–4. *Leotia lubrica*. 1. Ascocarp, approx. × 0.5. 2. Longitudinal section of an ascocarp showing nongelatinous middle layer stained dark, approx. × 10. 3. Ascospores, × 900. 4. Ascus, × 900. FIG. 5. *Leotia atrovirens*, ascocarps, approx. × 1. FIG. 6. *Leotia viscosa*, approx. × 0.8. FIG. 7. *Leotia albiceps*, ascocarps, approx. × 2. (Photograph by H. S. Jackson.)

mella of the Ombrophiloideae of the Helotiaceae sensu Nannfeldt. *Leotia* differs from the latter genera in having well developed stipes and pileate ascocarps.

Vibrissea is distinguished from other genera of the Geoglossaceae by the long filiform ascospores (FIG. 12). Nannfeldt (**1932**) placed this genus in the Ostropaceae of the Ostropales. It, however, does not have asci with walls hemispherically thickened at the apices and fragmentation of ascospores which are given as characteristic of that family. There is considerable variation in the shape of ascospores in the Geoglossaceae and the genus is retained. *Apostemidium* is included in the Geoglossaceae by Durand. It has filiform spores like *Vibrissea* but the ascocarps are sessile and pulvinate and therefore it is excluded.

Cudonia is closely related to *Spathularia* in the Geoglosseae. It has similar ascospores, which in some species produce conidia on sterigmata (FIG. 18), as in some species of *Spathularia*. *Cudonia lutea*, like *Spathularia velutipes*, has the hymenium covered by a well developed membrane until late in the development of the ascocarp (FIG. 16). On the other hand, *Cudonia* is related to *Helotium*. As has been discussed elsewhere (Mains **1956**) the type species of *Cudoniella* (*Cudoniella queletii* = *Helotium aciculare*), which has been included in the Geoglossaceae (Schroeter **1897**, Rehm **1895**, Clements and Shear **1931**), is the same as that for *Helotium*. Except for size, the ascocarps of *Helotium aciculare* are very similar to those of *Cudonia* in form and structure. The principal distinction is in fusoid ascospores.

Corner (**1930**), in his study of the evolution of the ascocarp in the Discomycetes, has concluded that "*Cudoniella acicularis*" occupies an intermediate position in the series between the Helotiaceae and the Geoglossaceae. Only relatively few of the many species which have been included in *Helotium* are similar to *Helotium aciculare*. As discussed elsewhere (Mains **1956**) the inclusion of *Helotium* (*Cudoniella*) as typified by *H. aciculare* in the Geoglossaceae would involve extensive studies concerning the limitations of the genus *Helotium*, the relation of genera in the Helotiaceae and probably a realignment of the genera of the Helotiaceae and Cudonieae. In this study it is not included and the Cudonieae as recognized by Durand has been followed with modifications as discussed. *Helotium* has, however, been included in the key for convenience.

KEY TO THE GEOGLOSSACEAE

A. Ascocarps clavate, spathulate or capitate, the hymenium covering most or all of the head or the upper part of the club.....**Geoglosseae**
(see Mains **1954**, **1955**)

A. Ascocarps pileate, the pilei convex, the hymenium covering the upper surface, the lower surface sterile.....**Cudonieae**

CUDONIEAE

1. Ascocarps gelatinous.....*Leotia*
1. Ascocarps fleshy.....2
2. Ascospores filiform.....*Vibrissea*
2. Ascospores acicular or narrowly clavate.....*Cudonia*
2. Ascospores fusoid.....*Helotium* (*Cudoniella*)
(see Mains **1956**)

LEOTIA Fr. Syst. Myc. **2**: 25. 1822

Ascocarps pileate, stipitate, gelatinous, with an outer gelatinous layer separated from an inner gelatinous center by a layer of non-gelatinous hyphae; hymenia covering the upper surface of the convex pilei; asci clavate, —I, 8-spored; ascospores subfusoid or narrowly ellipsoid, usually asymmetric, mostly multiguttulate, hyaline; paraphyses filiform, branched.

Type: *Leotia lubrica* Fr.

As treated by Fries in the Systema, *Leotia* was divided into two sections, *Hygromitra* having gelatinous ascocarps and *Cucullaria*, with fleshy ascocarps, including *Leotia circinans*. He later (**1849**) limited the genus to the gelatinous section and the genus *Cudonia* was established to which *Leotia circinans* was transferred.

Durand treats three species for North America. They are closely related, differing mostly in color of the ascocarps, and show considerable intergradation. Imai (**1941**) has considered them as forms of *Leotia lubrica* and has recognized eight additional forms differing mostly in size and color. Since the color is often considerably changed by drying, accurate determination of dried specimens is frequently impossible.

Durand and Imai state that the ascospores of species of *Leotia* are at first continuous, finally 3–5-septate. In the specimens examined in this study, the discharged ascospores are usually multiguttulate (FIG. 3) as in *Microglossum*. Septate spores were not seen.

The structure of the ascocarps is distinctive (FIGS. 2, 8). The central core of the stipe is made up of interwoven hyphae having narrow lumina and very gelatinous walls. Although these hyphae grow in various directions, the longitudinal direction is the most pronounced. At the upper end of the stipe they diverge outward and make up the medulla of the pileus. Surrounding the central gelatinous core of the stipe is a cylinder of compact non-gelatinous longitudinal hyphae. Above they spread outward and form a middle layer between the

medulla and the ectal layer on the lower side of the pileus. The outer layer of the stipe consists of gelatinous hyphae similar to those of the central core. Although they are very interwoven, they appear to arise from the non-gelatinous layer and develop outwardly. A continuation of the outer layer forms the ectal layer of the lower side of the pileus.

The structure of the ascocarps of *Leotia* relates it to species of the Ombrophiloideae of the Helotiaceae sensu Nannfeldt. According to von Höhnel (**1918**) the ascocarps of *Ombrophila violacea*, the type of *Ombrophila*, have a structure similar to that which has been described here for *Leotia*. The ascocarps of *Ombrophila* are substipitate to short-stipitate and the hymenia plane, plano-convex or somewhat concave. It should be noted that the ascocarps of *Ombrophila clavus*, which Seaver recognizes as the type of *Ombrophila*, do not have this structure. Von Höhnel, however, excludes *O. clavus* from *Ombrophila* and suggests that it belongs in *Helotium*. *Ascotremella*, placed by Nannfeldt in the Ombrophiloideae, also has ascocarps having an outer and inner gelatinous tissue separated by a non-gelatinous layer. The ascocarps are sessile to substipitate and the hymenia plane to convex. *Leotia* differs therefore principally in having well-developed stipes and more convex hymenia. This is also true for *Ombrophila albiceps* Peck. The ascocarps of this species also have the structure of *Leotia* and the species is therefore transferred here to *Leotia*.

KEY TO THE SPECIES OF LEOTIA

1. Ascospores 5–7.5 × 2–3 μ.....*L. albiceps*
1. Ascospores 16–25 × 4–6 μ.....2
2. Ascocarps buff, ochraceous or cinnamon, sometimes olivaceous.....*L. lubrica*
2. Ascocarps dark green, sometimes with stipe light green.....*L. atrovirens*
2. Ascocarps with the hymenium dark green and the stipe and lower surface of the pileus white, yellow or orange.....*L. viscosa*

LEOTIA LUBRICA Fr. Syst. Myc. **2**: 29. 1822. FIGS. 1–4

Leotia punctipes Peck, Bul. Torrey Bot. Club **34**: 102. 1907.

Ascocarps cespitose, gregarious or sometimes scattered, 2–7 cm long, pileate, gelatinous or under dry conditions appearing somewhat fleshy, buff, ochraceous or cinnamon, sometimes with a greenish tinge or olivaceous; pilei convex, smooth or somewhat furrowed or wrinkled above, squamulose below, 8–40 mm broad consisting of three layers continuous with those of the stipe; stipes terete, equal or somewhat enlarged below, 5–10 mm thick, minutely squamulose or furfuraceous, consisting of three layers, a central core of interwoven gelatinous hyphae, a middle layer of non-gelatinous longitudinal hyphae and an outer layer

of gelatinous hyphae; asci clavate, 115–150 × 7–10 μ; ascospores subfusoid, narrowly ellipsoid, straight or slightly curved, rounded at the ends, 16–23 × 4–6 μ, multiguttulate; paraphyses filiform, somewhat enlarged at the apices, branched below, usually somewhat agglutinated with amorphous matter.

On soil or sometimes on rotting wood. Collected in Michigan from July 9 to October 25.

Specimens studied: 88 from California, Kentucky, Maine, Maryland, Massachusetts, Michigan, New Jersey, New York, North Carolina, Ohio, Oregon, Pennsylvania, Tennessee, Vermont, Virginia, Washington, Nova Scotia, Ontario, Quebec (all MICH).

Leotia lubrica is the most abundant species in North America as well as elsewhere. It is very variable in size and through the greenish variants intergrades into *L. viscosa* and *L. atrovirens*. Durand recognized two forms, *L. lubrica* f. *stevensoni* having greenish or olivaceous ascocarps with firm consistency and *L. lubrica* f. *lloydii* having olive-ochraceous ascocarps with firm consistency which on drying become darker green or olive with olive-green stipes.

LEOTIA ATROVIRENS Pers. ex Fr. Syst. Myc. **2**: 30. 1822. FIG. 5

Leotia chlorocephala Schw. ex Fr. Syst. Myc. **2**: 30. 1822.

Ascocarps scattered or cespitose, 0.5–4 cm long, pileate, gelatinous or subgelatinous, similar in structure to *L. lubrica;* pilei convex, 3–10 mm wide, 2–5 mm thick, dark green, squamulose below; stipes terete, 2–4 mm thick, concolorous with pilei or lighter green, usually prominently squamulose; asci clavate, 125–150 × 8–10 μ; ascospores subfusoid, straight or somewhat curved, 16–22 × 4–5 μ, multiguttulate; paraphyses filiform, somewhat enlarged at the apices, branched below, green above, somewhat agglutinated with green amorphous matter.

On soil. Collected in Michigan from Aug. 10 to Sept. 11.

Specimens studied: 19 from Florida, Massachusetts, Michigan, New Hampshire, New York, North Carolina, Ontario, Quebec (all MICH).

This species differs from *L. lubrica* in its definitely green color, smaller size, firmer consistency and more prominently squamulose condition. It is treated by Durand under the name *L. chlorocephala* Schw. Both Durand and Nannfeldt (**1942**) have noted the close similarity to *L. atrovirens* of Europe. They do not appear to differ sufficiently to justify the recognition of two species. There is some question concerning the name which should be employed. Both are published by Fries in 1822 on the same page of his Systema. Fries refers to the

publication of *L. chlorocephala* by Schweinitz in his Synopsis Fungorum Carolinae published in 1822 and to the publication of *L. atrovirens* by Persoon in his Mycologia Europaea pt. 1 also published in 1822. Rogers (**1944**) has concluded that Schweinitz's Synopsis Fungorum Carolinae was published later in 1822 than Persoon's Mycologia Europaea. Consequently *L. atrovirens* appears to have precedence over *L. chlorocephala*. *L. stevensoni* Berk. & Br. may also be this species. Massee (**1897**) and Durand, however, consider it to be a greenish form of *L. lubrica*.

LEOTIA VISCOSA Fr. Syst. Myc. **2**: 30. 1822. FIG. 6

Leotia stipitata Schroeter. Engl. Prantl. Nat. Pfl. **1**: 166. 1894.

Ascocarps cespitose or scattered, 3–9 cm long, pileate, gelatinous, similar in structure to *L. lubrica;* pilei convex, 2–3 cm wide, 5–10 mm thick, olive-green to dark green; stipes terete or somewhat flattened, 5–10 mm thick, white, yellow or orange, green-punctate or furfuraceous; asci clavate, 125–160 × 8–11 μ; ascospores subfusoid to narrowly ellipsoid, straight or slightly curved, rounded at the ends, 17–26 × 4–6 μ, multiguttulate; paraphyses filiform, branched below, somewhat enlarged at the apices, green above, usually somewhat agglutinated with green amorphous matter.

On soil or sometimes on rotten wood. Collected in Michigan from July 24 to November 13.

Specimens studied: 43 from Massachusetts, Michigan, New Hampshire, New York, North Carolina, Oregon, South Carolina, Tennessee, Virginia, Nova Scotia, Ontario, Quebec (all MICH).

This species was treated by Durand as *L. stipitata*. It is very similar to *L. lubrica* and is distinguished from it by the dark green pilei and white to orange stipes. It might be desirable to recognize varieties based on the color of the stipes.

Leotia albiceps (Peck) comb. nov. FIGS. 7–9

Ombrophila albiceps Peck Ann. Rep. N. Y. State Mus. **42**: 34. 1889.

Ascocarps solitary, gregarious or cespitose, 5–30 mm long, pileate, stipitate, gelatinous with gluten often covering stipe and lower surface of the pileus, drying horny, structure similar to *L. lubrica;* pileus hemispherical, 3–12 mm broad, 2–3 mm thick, watery white or carneus at first, becoming ochraceous to army brown, with the hymenium convex on the upper surface, with the margin becoming recurved; stipe terete, 2–4 mm thick below, above widening into the pileus, smooth or rough through irregular drying of the gluten, brown with lilac tinge; asci

clavate, 40–60 × 4–6.5 μ; ascospores subfusoid to narrowly ellipsoid, 5–7 × 2–3 μ, guttulate; paraphyses linear, slightly exceeding the asci.

TYPE: On decaying wood, North Elba, N. Y., Sept., Charles H. Peck (NYS).

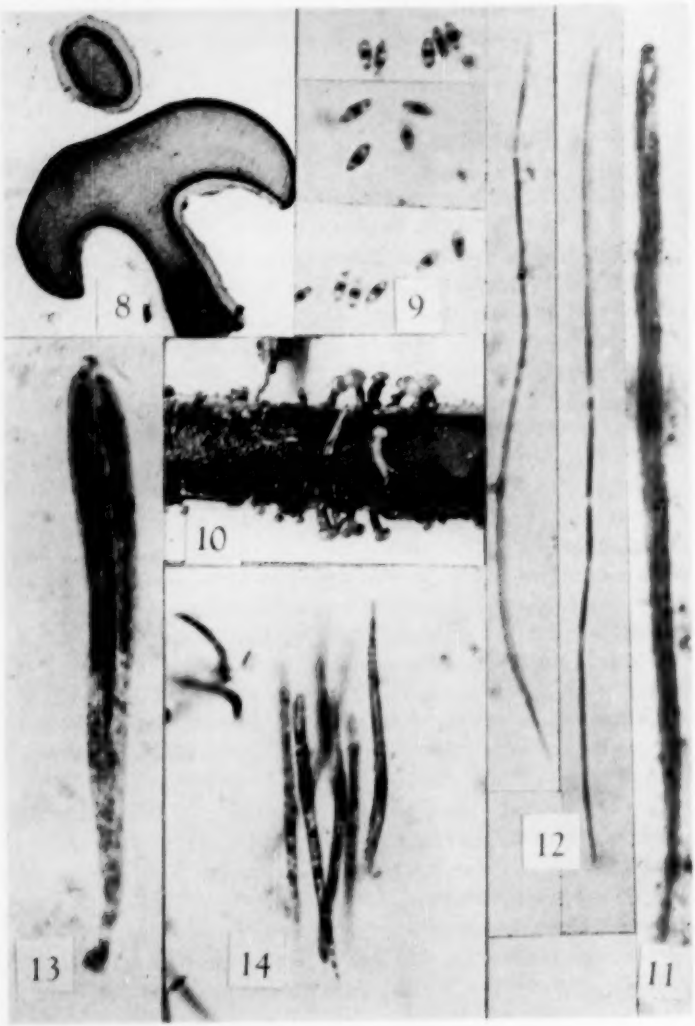
On rotting poplar wood.

Specimens studied: Michigan E. B. & E. E. Mains, *33–658* (MICH). New York, C. H. Peck, TYPE (NYS). Ontario, H. S. Jackson *et al*, TRT *3082, 5936, 5937, 5938, 5939, 5940, 5960, 7953, 7955, 7956, 9711.*

In correspondence with the late Professor H. S. Jackson, he suggested that the Michigan collection might be *Ombrophila albiceps* Peck and stated that he had collected it in Ontario. He was doubtful whether it was a species of *Ombrophila* or *Leotia* and noted a resemblance in the young stages to *Ascotremella turbinata.*

Through the kindness of Roy C. Cain, the specimens in the Herbarium of the University of Toronto were loaned for study. They are 11 ample collections with notes by Professor Jackson. He described the color of pilei of the fresh ascocarps as "pearly or watery white at first often with flesh tints becoming ochraceous" and the stipes as brownish, often with a lilac tinge. A study of sections of the ascocarps showed a structure similar to that found in *L. lubrica* (Fig. 8). The central core of the stipe consists of longitudinal interwoven hyphae with very gelatinous walls. Upwards these hyphae spread outward to form the medulla of the pileus. Surrounding this is a layer of compact longitudinal non-gelatinous hyphae which continues on the underside of the pileus. Apparently arising from this layer and extending outward are gelatinous hyphae which form an outer gelatinous layer of the stipe and underside of the pileus. Jackson mentions an outer layer of gluten in his notes and suggests that it is in the nature of a universal veil. It probably is due to the excessive gelatinization of the walls of the hyphae of the outer layer. The Michigan specimen has a similar structure. It was army brown in color when collected.

Through the kindness of Stanley Jay Smith, the type of *Ombrophila albiceps* in the Herbarium of the New York State Museum was loaned for study. It consists of several ascocarps up to 15 mm long with pilei up to 8 mm broad, with well developed stipes. They are dark reddish brown. Peck described the pilei of the fresh ascocarps as "whitish or sometimes with a faint incarnate tinge" and the stipes as "pallid or reddish brown" appearing covered with gluten in wet weather. The asci are clavate, 50–55 × 4 μ, and the ascospores 5–6 × 2–2.5 μ. In section the ascocarps have two gelatinous layers separated by a layer of non-



FIGS. 8, 9. *Leotia albiceps*. 8. Longitudinal section of ascocarp (below) and cross section of stipe (above) showing nongelatinous middle layer stained dark, approx. × 10. 9. Fourteen ascospores, × 900. FIGS. 10–12. *Vibrissea foliorum*. 10. Ascocarps, approx. 0.5. 11. Ascus, × 900. 12. Two filiform ascospores, × 900. FIGS. 13, 14. *Cudonia circinans*. 13. Ascus, × 900. 14. Ascospores, × 900.

gelatinous hyphae as described for the Ontario specimens. The Michigan and Ontario specimens are therefore *Ombrophila albiceps*. However, its well developed stipe, convex hymenium and structure similar to *L. lubrica* place it in *Leotia*.

Imai (**1941**) has described a species *Neocudoniella jezoensis* from Japan with gelatinous ascocarps which from his discussion appears somewhat similar to *L. albiceps*. Information concerning the structure of the ascocarps is not given.

VIBRISSEA Fr. Syst. Myc. **2**: 31. 1822

Ascocarps pileate, stipitate, fleshy or subgelatinous; pilei convex, with the margins often involute, with hymenia on the upper surfaces; stipes terete, well developed; asci subcylindric to narrowly clavate, narrowing to the base, —I; ascospores 8, filiform, multiseptate, hyaline; paraphyses filiform, enlarged at the apices, simple or branched.

Type: *Vibrissea truncorum* Fr.

This genus is distinguished by its long, filiform, multiseptate ascospores (FIG. 12). Nannfeldt (**1932**) places it in the Ostropaceae, apparently mostly on account of its filiform ascospores. Since there is great variation in the ascospores of the Geoglossaceae, this is not sufficient to exclude it from the family. The ascospores of *Vibrissea* do not break up into part spores and the asci do not have a pronounced hemispherical thickening of the apical wall as in the Ostropaceae.

The hyphae of the interior of the stipes are hyaline, compact and longitudinal. There is usually no well differentiated outer layer. The outer hyphae are somewhat wider and sometimes brownish. The outermost hyphae may become free to form hairs or clumped to form scales. The hyphae diverge into the pileus, becoming compactly interwoven in the medulla. The outer hyphae of the stipe continue to form a layer of parallel hyphae on the underside of the pileus, diverging somewhat at the margin.

VIBRISSEA TRUNCORUM Fr. Syst. Myc. **2**: 31. 1822. FIGS. 10–12
Vibrissea foliorum Thaxter, in Durand, Ann. Myc. **6**: 454. 1908.

Ascocarps gregarious, cespitose or scattered, pileate, stipitate, fleshy to subgelatinous, 3–20 mm high; ascogenous portion convex, thin, 2–5 mm wide, flesh-colored, pinkish buff or yellowish buff, with hymenium on the upper surface, sterile underneath, with margin frequently involute; stipes terete, 1–2 mm thick, white, minutely tomentose, frequently matted and appearing smooth; asci narrowly clavate, gradually narrow-

ing from near the apex, variable in length, 175–325 × 5–6 μ; ascospores filiform, very variable in length (80–)125–250(–275) × 1 μ, multiseptate; paraphyses straight, simple or branched, filiform below, enlarged at the apices.

On decaying wood, leaves etc. in cold wet places, often in streams. Collected in Michigan from June 13 to August 6.

Specimens studied: 27 from Connecticut, Michigan, New Hampshire, New York, Washington, Ontario, Quebec (all MICH).

This is a species which develops under cold wet conditions. It often fruits early in the year submerged in cold running water where the long ascospores projecting above the hymenium vibrate in the stream and give it a white silky appearance.

Durand distinguished *V. foliorum* from *V. truncorum* on differences in habitat and sizes of asci and ascospores. The asci of *V. truncorum* were described as 200–325 × 5–6 μ and the ascospores as 175–250 × 1 μ and it is stated that the species develops on wholly or partly submerged sticks in brooks mostly in high altitudes. *V. foliorum* was described from one specimen on dead oak leaves, acorn cups etc. in a wet place and the asci are given as 150–180 × 5–6 μ and the ascospores as 80–100 × 1 μ. A study of part of the type collection (Rel. Farlowiana *175*) has resulted in finding asci 150–200 μ long and ascospores 80–135 μ.

In other collections examined in this study, considerable differences in ascospore length were found. Although collections from submerged wood tend to have longer spores than those on leaves and sticks in wet places, collections with asci up to 300 and ascospores up to 200 and 250 μ have been seen from the latter. Also in submerged collections short ascospores (up to 135 and 150 μ) occur. It therefore seems desirable to recognize only one species.

CUDONIA Fr. Summa Veg. Scand. 348. 1849

Ascocarps pileate, stipitate, fleshy or fleshy-leathery, pilei usually convex, with the hymenium on the upper surface, sterile beneath, with the margin often involute; asci clavate, —I, 8-spored; ascospores acicular or narrowly clavate, hyaline, 1-celled or multi-septate; paraphyses filiform, hyaline.

Type species: *Cudonia circinans* Fr.

Durand treats three species of *Cudonia* for North America, *C. circinans*, *C. lutea* and *C. ochroleuca*. The identity of the last species is very uncertain. It was described by Cooke and Harkness from a specimen collected at San Rafael, California. Durand states that the color

of the dried specimen is very different from that given in the description. Specimens which have been identified as *C. ochroleuca* have been *Cudonia monticola* and the lichen *Bacomyces roseus*. Since Durand's monograph, two other species have been described, *C. monticola* Mains and *C. grisea* Mains. Imai (**1955**) has recently proposed a genus *Pachycudonia* which he distinguishes from *Cudonia* by "long-tailed" asci, the spores being constricted at the middle portion and not perfectly acicular, and the circinate paraphyses. He includes *C. constrictospora*, *C. spathulata* and *C. monticola*. *C. monticola* has asci attenuated below (FIG. 19), but this occurs to some extent in other species, such as *C. lutea* (FIG. 17). The ascospores of *C. monticola* (FIG. 20) are not constricted or only rarely so. Throughout the genus *Cudonia* the paraphyses are strongly curved, uncinate or circinate above. It seems doubtful that a generic separation is justified. In this study (Mains **1955**) *C. spathulata* has been included in *Spathularia*.

The development of *Cudonia lutea* has been studied by Duff (**1922**). An outer layer of tissue develops early in the growth of the ascocarp. This later becomes the veil which completely covers the ascocarp. The hymenium develops beneath the veil on the upper surface of the young pileus. With the expansion of the young pileus the covering veil breaks and sloughs off except for a few adhering remnants (FIG. 16). Portions of the veil are usually found in most collections of *C. lutea*. Whether a veil is formed in other species of the genus is unknown. If formed, it probably is poorly developed and evanescent. The ascospores of *Cudonia* are usually one-celled. Septa are sometimes formed and the spores then may become 2- to several-celled. In *C. circinans* and *C. lutea* the ascospores commonly produce conidia on short sterigmata. When this occurs within the asci, the conidia may entirely replace the ascospores.

Cudonia appears to be very closely related to *Spathularia* of the Geoglosseae. The two genera have a parallel series of species differing in types of asci, formation of conidia and development of veil over the hymenium.

KEY TO THE SPECIES OF CUDONIA

1. Ascospores 18–25 μ long 2
1. Ascospores mostly more than 30 μ long 3
2. Pilei drab or dark gray, stipes fuscus ..... *C. grisea*
2. Pilei pinkish cinnamon or pinkish buff, stipes avellaneous or wood brown ..... *C. monticola*
3. Ascospores (28–)32–40(–46) μ long ..... *C. circinans*
3. Ascospores (48–)50–65(–70) μ long ..... *C. lutea*

CUDONIA CIRCINANS Fr. Summa Veg. Scand. 348. 1849. FIGS. 13, 14
Leotia circinans Fr. Syst. Myc. **2**: 27. 1822.

Ascocarps gregarious or cespitose, pileate, stipitate, fleshy, drying leathery, 1.5–7 cm long; pileus thin, up to 2 cm broad, convex, smooth, wrinkled or sometimes convoluted, cream to dark brown, with the hymenium on the upper surface, with the lower surface sterile, with the margin often involute; stipe terete, 2–12 mm thick, furfuraceous, striate to ridged, drab to dark brown; asci clavate, 90–150 × 8–10 μ; ascospores acicular (28–)32–40(–46) × 2 μ, 1-celled or sometimes several-septate, the wall thin, gelatinous; conidia commonly produced on short sterigmata by the ascospores, subspherical to broadly ellipsoid, 3–4 × 2 μ, hyaline, sometimes replacing the ascospores in the asci; paraphyses filiform, branched below, strongly curved or uncinate above, hyaline.

On soil, less commonly on rotting wood. Collected in Michigan from August 4 to October 3.

Specimens studied: 99 from Colorado, Idaho, Michigan, New York, Oregon, Tennessee, Washington, Nova Scotia (all MICH).

This is the most common species in North America. It varies considerably in color. In the fresh condition the pileus may be cream, pinkish buff, cinnamon-buff, vinaceous buff, avellaneous or wood brown and the stipe usually darker, drab, vinaceous brown, wood brown or bone brown. Fries commented on the variation in color of this species.

The central portion of stipe and pileus consists of very loosely interwoven hyphae and may become hollow in the larger ascocarps. The hyphae of the outer portion of the stipe and lower side of the pileus are compact more or less parallel to the surface. The furfuraceous condition is due to groups of short branches arising from the outer hyphae and consisting of short ellipsoid to oblong cells.

Nannfeldt (**1942**) has followed Bresadola in recognizing a species *C. confusa* in Europe which is distinguished from *C. circinans* mostly by color.

CUDONIA LUTEA (Peck) Sacc. Atti Real. Inst. Venet. **6**: 725. 1885. FIGS. 15–18
Vibrissea lutea Peck, Rep. N. Y. State Mus. **25**: 97. 1873.
Leotia lutea Cooke, Bul. Buffalo Soc. Nat. Sci. **2**: 287. 1875.

Ascocarps gregarious or scattered, pileate, stipitate, fleshy, drying leathery, 1–6 cm long; pileus up to 1.5 cm broad, convex above, margin often involute, upper surface smooth, frequently with portions of the veil adhering to the margin or as patches on the surface, yellowish, orange-buff, ochraceous or ochraceous buff, the lower surface of pileus

furfuraceous, often with ridges continuing down the stipe; stipe terete, up to 6 mm thick, concolorous with the pileus or lighter, minutely furfuraceous; asci clavate, 110–160 × 10–12 μ; ascospores acicular (48–) 50–65(–70) × 2 μ, 1-celled or multiseptate, wall thin, with an outer gelatinous layer; conidia frequently produced on short sterigmata by the ascospores, subspherical to broadly ellipsoid, 3–4 × 2 μ, hyaline, sometimes replacing the ascospores in the asci; paraphyses filiform, branched below, strongly curved to circinate above, hyaline.

TYPE: North Elba, Essex Co., N. Y., Charles Peck in the Herbarium of the N. Y. State Museum.

On decaying leaves usually of beech. Collected in Michigan from July 30 to October 7.

Specimens studied: 33 from Michigan, New Hampshire, New York, North Carolina, Ohio, West Virginia, Vermont, Nova Scotia, Quebec (all MICH); also the type from N. Y. S. Museum.

The ascospores of *C. lutea* are larger than those of *C. circinans*. Similarly the wall gelatinizes and they commonly produce conidia (FIG. 18).

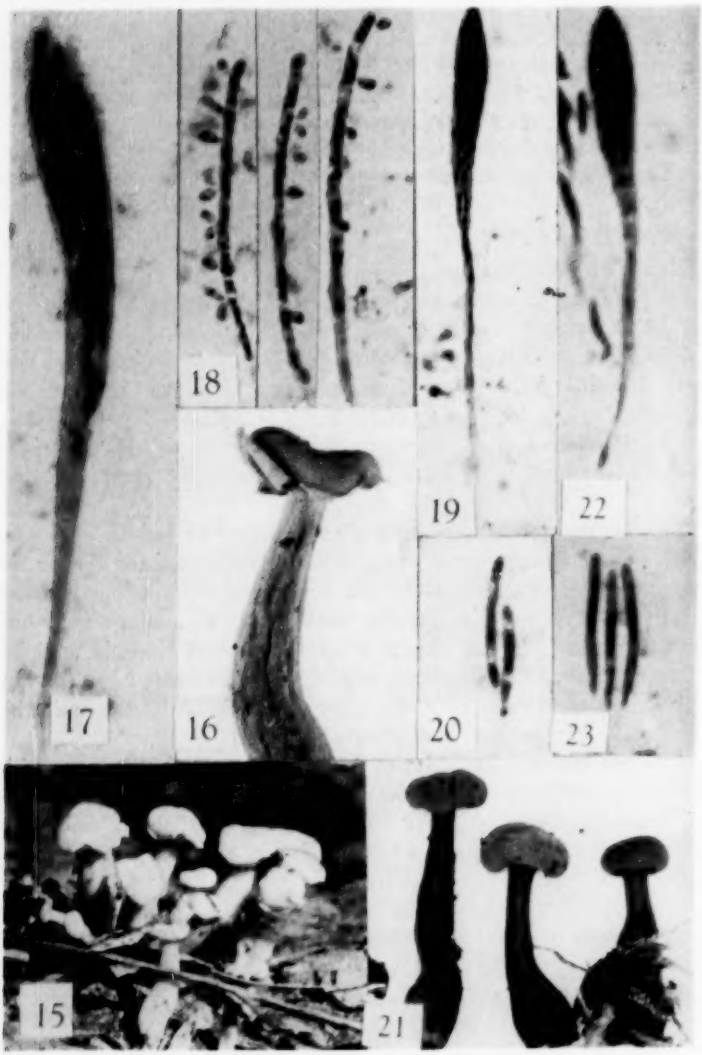
The interior of the stipe and pileus consists of loosely interwoven hyphae which become more compact and parallel toward the outside. The outer layer of the stipe and the lower side of the pileus is a compact tissue of densely interwoven cells which in sections is pseudoparenchymatous. On the outside it forms clumps of ellipsoid to globoid cells which give the furfuraceous condition. This outer layer continues in the young condition over the hymenium and forms the veil which later breaks into patches and sloughs off.

Cudonia helvelloides S. Ito & Imai in Japan is described as having ascospores similar in size, 48–60 × 1.5–2 μ. The pilei are at first convex, becoming helvelloid, and are at first pale yellowish, finally pale avellaneous. *C. japonica* Yasuda of Japan has much larger spores 65–85 × 2.5–3.5 μ.

CUDONIA MONTICOLA Mains, Am. Jour. Bot. **27**: 322. 1940. FIGS. 19, 20

Pachycudonia monticola Imai, Sci. Rep. Yokohama Nat. Univ. Ser. 2, no. 4: 2. 1955.

Ascocarps closely gregarious to cespitose, pileate, stipitate, fleshy-leathery, 3–10 cm long; pilei 1–3 cm broad, convex, irregularly hemispherical, laterally compressed or helvelloid, rugose, pinkish cinnamon, pinkish buff or grayish brown; stipe somewhat compressed, 5–7 mm thick below, somewhat narrowed above, becoming hollow in age, pallid



FIGS. 15–18. *Cudonia lutea*. 15. Ascocarps, approx. × 1. 16. Ascocarp showing fragments of veil at margin of hymenium, approx. × 2. 17. Ascus, × 900. 18. Three ascospores with conidia, × 900. FIGS. 19, 20. *Cudonia monticola*. 19. Ascus, × 900. 20. Two ascospores, × 900. FIGS. 21–23. *Cudonia grisea*. 21. Ascocarps, approx. × 1. 22. Ascus, × 900. 23. Three ascospores, × 900.

avellaneous or wood brown, glabrous; asci clavate, 90–125 × 8–10 μ, narrowing rapidly below the upper third, becoming 2–4 μ thick below; ascospores acicular or narrowly clavate (15–)18–24(–28) × 2 μ, 1-celled or rarely 1-septate; paraphyses filiform, strongly curved to uncinate above, hyaline.

TYPE: Lake Crescent, Washington. A. H. Smith *14060*, in the Herbarium of the University of Michigan.

On soil, coniferous debris or rotting wood.

Specimens studied: California, CU *521118*; Idaho, A. H. Smith *45210, 45224* (MICH); Washington, A. H. Smith *13785, 14009, 14060*, TYPE (MICH); Wm. B. Gruber, June 22, 1942 (MICH).

This is the largest species in North America. It has much smaller ascospores (FIG. 20) than either *C. circinans* or *C. lutea*. The structure of the ascocarp is similar to *C. circinans*.

Cudonia osterwaldii P. Henn. in Germany is described as having small, 0.5–1.5 cm, ascocarps with dark chestnut colored pilei and pallid stipes. The ascospores are narrowly clavate, 18–28 × 2–4 μ, and multiguttulate. *Cudonia constrictospora* S. Ito & Imai in Japan is described as having yellowish, pale isabelline or subochraceous ascocarps and ascospores which are narrowly clavate, 20–27.5 × 2 μ and constricted in the middle.

CUDONIA GRISEA Mains, Am. Jour. Bot. **27**: 322. 1940. FIGS. 21–23

Ascocarps gregarious, pileate, stipitate, fleshy, 1.5–5 cm long; pilei 0.5–1.5 cm broad, convex, smooth, drab or dark grey; stipe terete, 3–8 mm thick below, narrowing upward, smooth, fuscus; asci clavate, 70–110 × 6–10 μ, narrowing below the upper third, attenuated below; ascospores acicular 18–22(–24) × 1.5–2 μ, 1-celled or rarely 1-septate; paraphyses filiform, strongly curved above, hyaline.

TYPE: Hoh River, Washington, A. H. Smith *13521* in the Herbarium of the University of Michigan.

On rotting coniferous wood.

Specimens studied: Washington, A. H. Smith *13521*, TYPE, *14100, 14482* (all MICH).

Cudonia grisea has similar ascospores to those of *C. monticola*. The ascocarps are smaller and differ in color.

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DALEOMYCES, DURANDIOMYCES, AND OTHER SPARASSOID FORMS OF OPERCULATE DISCOMYCETES

Richard P. Korf[1]

I. The sparassoid form of Peziza proteana

A thoroughly confused nomenclatural and taxonomic problem in the Operculate Discomycetes revolves around a fungus which attains at times a truly immense size (not uncommonly 30 cm in diameter, and reported at least as large as 120 cm in diameter). It has been termed the "cabbage-head fungus," and in many ways it indeed resembles a head of cabbage. Several herbarium labels and notes refer to its excellent esculent qualities. The fruitbody has been amply, and often beautifully, illustrated (**1**, **3**, **4**, **5**: pl. 294, **7**, **17**, **19**, 22, 23, **24**).

The fungus has been described as belonging to two orders (Pezizales, Tuberales) and three families (Helvellaceae, Pezizaceae, Tuberaceae). It has been made the holotype of three genera (*Daleomyces, Durandiomyces, Napomyces*), and has been referred to five other genera (*Aleuria, Galactinia, Gyromitra, Peziza, Underwoodia*). This Discomycete is the basis of at least four specific epithets (*campbellii, gardneri, phillipsii, sparassoides*) and of at least two varietal epithets (*slavkoviensis, sparassoides*). I have had before me for study the type material of each of the epithets involved (except var. *slavkoviensis*), and all prove to be almost identical and certainly referable to a single species.

The white to pinkish cabbage-head fungus usually occurs on burned-over areas, not uncommonly on burned stumps and/or soil in beech or

[1] Acknowledgments: This study would have been impossible to pursue without the unfailing cooperation of the curators and directors of several herbaria. Permission to examine specimens in the Boudier herbarium and facilities for this were graciously provided by Prof. R. Heim and Dr. Marcelle Le Gal, Muséum National d'Histoire Naturelle, Paris. Specimens of great value were loaned by Dr. F. L. Balfour-Browne and Dr. G. Taylor, Keeper, British Museum (Natural History); Prof. L. Bonar, University of California; Prof. C. Cappelletti, Orto Botanico, Padova; Dr. Helen M. Gilkey, Oregon State College; Dr. D. P. Rogers, New York Botanical Garden. In addition Dr. S. Ahlner, Naturhistoriska Riksmuseum, Stockholm, and Dr. R. W. G. Dennis, Royal Botanic Gardens, Kew, provided information regarding specimens in their herbaria. Prof. R. T. Clausen, Cornell University, has been of assistance in nomenclatural problems.

predominantly beech forests. In association with the cabbage-head fungus, three investigators (Bánhegyi (**2**), Boudier (**3**), Durand (**7**)) have reported finding a small (2–6 cm diameter), similarly colored, cupulate to applanate Discomycete, *Peziza proteana*. In all anatomical and microchemical characters that I have been able to observe, this more common, cupulate form is identical with the cabbage-head fungus except for its simple structure.

The results of my investigations of the type specimens cited below, as well as over twenty additional collections of the cabbage-head fungus, tend to support fully the conclusions of Boudier (**3**, **4**, **5**), Durand (**7**), Le Gal (**15**), Neuwirth (**17**) and Bánhegyi (**2**), that it is merely an immense, compound form of *Peziza proteana*. In some specimens there is an undifferentiated, sterile base which has been termed by some authors a stipe. The large masses of tissue that make up the fertile portion of the cabbage-head fruitbody appear to consist of many simple apothecia fused together. Sections of the tissue between adjacent locules confirm this, for between the hymenial layers are separate hypothecial layers grading into typical excipular layers which appear to be fused, back to back. The fruitbody of the cabbage-head fungus can hardly be thought of as a single apothecium; it could be aptly termed a "compound ascocarp." Since it occurs regularly along with typical *P. proteana*, it seems highly likely that the cabbage-head form arises *de novo* under certain stimuli. There appears to be no evidence to indicate whether this change is genetically controlled.

Those authors (Setchell (**24**), Clements & Shear (**6**), Fischer (**8**), Gilkey (**9**)) who have referred the cabbage-head fungus to the Tuberales have felt that a peridium is present. In no case have I noted anything which might be analogous to a peridium other than typical excipular tissues of the outermost apothecia. The asci are operculate, and apparently functional, which would seem ample reason for removing the fungus from the Tuberales and placing it in the Pezizales.

The microscopic and microchemical characters of the cabbage-head fungus clearly exclude it from the Helvellaceae where it has been treated by several authors (Bánhegyi (**1**), Hone (**12**), Massee (**16**), Phillips (**19**), Saccardo (**21**), Seaver (**22**, **23**)). Ascus and spore characters leave no doubt in my mind but that the cabbage-head fungus should be placed in the genus *Peziza*. The ascus apex blues with iodine (at times very strongly) as is characteristic of the genus, and the marked spores provided with two guttules are characteristic of the subgenus *Galactinia* (treated by some authors as a separate genus), to which it should be referred. Except for certain differences in nomenclature,

this is the treatment afforded it by Bánhegyi (**2**), Boudier (**3**, **4**, **5**), Durand (**7**), Le Gal (**15**), Neuwirth (**17**) and Saccardo (**20**). *Daleomyces* Setchell, *Durandiomyces* Seaver and *Napomyces* Setchell ex Clements & Shear are thus all reduced to synonymy with *Peziza* Hooker subgen. *Galactinia* Cooke, since all three generic names have the cabbage-head fungus as their holotypes.

The ascospores of the cabbage-head fungus are characteristically marked with fairly medium-sized, usually discrete warts, which stain dark blue with cotton-blue dyes (**13**). The ascospores have been rather faithfully illustrated by several authors (**1**, **5**, **17**). One of the reasons this study was undertaken was because of the descriptions and illustrations of the ascospores of *Daleomyces gardneri* by Setchell (**24**) and by Gilkey (**9**). They draw and describe regularly reticulate, rather than verrucose, ascospores. My examination of the type and paratype specimens of *D. gardneri* from which their drawings were made have shown these to have verrucose spores. In water and in iodine mounts, however, when the spore markings are not stained, the regular placement of the warts on the spore can easily give rise to the illusion that the spores are reticulate, for the unmarked areas *between* the warts simulate a reticulum. It is not difficult to see how both Setchell and Gilkey made their errors, since neither had a spore-marking stain at their disposal.

The application of Article 77 of the 1952 International Code (**14**) appears to offer some difficulty. This article states that names and epithets based on a "monstrosity" must be rejected. Can the cabbage-head fungus be termed a monstrosity? If so, *all* of the names and epithets given to the sparassoid form must be rejected, and the correct name to use would be *Peziza proteana* for the combined species containing a cupulate and a sparassoid form. *No* infraspecific epithet could be used to distinguish these two, grossly dissimilar forms. A major problem still remains, however, as to which condition, cupulate or sparassoid, is "normal" for the fungus, or indeed whether or not both may be "normal." I believe it has been fully illustrated that the sparassoid form has been, and will continue to be, distinguished by mycologists and accorded taxonomic identity. Article 77 is so vague as to make its application in this case questionable and presumably inoperative. The names and epithets based on the sparassoid form thus appear to need consideration from a nomenclatural standpoint. The oldest specific epithet for either form is apparently *phillipsii*, which happens to be based on the sparassoid form, and the combined species should bear this epithet. There exists, however, an earlier homonym which would

prevent the transfer of Massee's epithet to the genus *Peziza*, namely *Peziza phillipsii* Cooke, 1879. The next oldest available epithet appears to be *proteana*, which happens to be based on the cupulate form, and when treated under the genus *Peziza* this epithet must be used.

Evidence pointing to the origin of the cabbage-head fungus at various times directly from the cupulate form of *P. proteana* (or, with equal possibility, *vice versa*) leaves no doubt in my mind that the two entities should be treated as minor, infraspecific taxa of one species. Varietal status (as well as specific and generic status!) has been proposed in the past, but I feel to term these more than forms is to overemphasize their biological permanence and stability. I elect (as permitted under the Code, **14**: Art. 70) to use Boudier's varietal epithet rather than Massee's earlier specific epithet for transfer and typification on the basis that the resulting name is far more descriptive. The sparassoid form thus becomes:[2]

PEZIZA (subgen. GALACTINIA) PROTEANA (Boud.) Seaver forma **sparassoides** (Boud.) Korf, comb. nov.

= *Gyromitra phillipsii* Massee, Br. Fungus-Fl. **4**: 478. 1895. (!!), non *Peziza phillipsii* Cooke (ut "phillipsi"), Mycographia p. 48. 1879.

= *Durandiomyces phillipsii* (Massee) Seaver, N. Am. Cup-fungi (Operculates) p. 242. 1928.

= *Daleomyces phillipsii* (Massee) Seaver, N. Am. Cup-fungi (Operculates), suppl. ed. p. 337. 1942.

= *Aleuria* (*Galactinia*) *proteana* Boud. var. *sparassoides* Boud., Bull. Soc. Myc. Fr. **15**: 51. 1899. (!!)

= *Galactinia proteana* (Boud.) Sacc. & Syd. *in* Sacc. var. *sparassoides* (Boud.) Sacc. & Syd. *in* Sacc., Syll. Fung. **16**: 709. 1902.

= *Peziza proteana* (Boud.) Seaver var. *sparassoides* (Boud.) Durand, Mycologia **11**: 1. 1919.

= *Underwoodia sparassoides* (Boud.) Bánhegyi, Index Horti Bot. Univ. Budap. **3**: 19. 1937.

= *Underwoodia campbellii* Sacc. (ut "campbelli"), Ann. Myc. **7**: 433. 1909. (!!)

[2] The identity sign (=) indicates nomenclatural synonymy (obligate); the equality sign (=) indicates taxonomic synonymy. The symbol (!!) indicates holotype or isotype material examined, and the symbol (!) indicates paratype material examined. Herbarium abbreviations: BM-P = Phillips Herbarium, British Museum (Natural History); NY = New York Botanical Garden; PAD-S = Saccardo Herbarium, Padova; PC-B = Boudier Herbarium, Muséum National d'Histoire Naturelle, Paris; UC = University of California Herbarium.

= *Daleomyces gardneri* Setchell, Mycologia **16**: 241. 1924. (!!) (!)
= *Napomyces gardneri* (Setchell) Setchell ex Clem. & Shear, Gen. Fungi p. 333. 1931.
= *Aleuria proteana* Boud. var. *slavkoviensis* Neuwirth, Studia Bot. Čechosl. **7**: 172. 1946.

TYPE SPECIMENS EXAMINED:

England: "Gyromitra gigas. Mrs. Coker Beck," BM-P, HOLOTYPE of *Gyromitra phillipsii* Mass.

France: "Galactinia Proteana Boud. Monstrosa. *forma* sparassoides Boud. in carbonariis. Trilport propè Meaux. 8br 1898," PC-B, HOLOTYPE of *Aleuria proteana* var. *sparassoides* Boud.

Italy: "Ad truncos fagineos vetustos—Edulis—Prope Sora, Jun. 1909 (Campbell)," PAD-S, HOLOTYPE of *Underwoodia campbellii* Sacc.

United States: "In grass under Eucalyptus, Univ. of California campus, Berkeley, Alameda Co., Dale & H. E. Parks *1412*, Jan. 22, 1923, part of type specimen," UC, HOLOTYPE of *Daleomyces gardneri* Setchell; *Ibid.*, NY, ISOTYPE; "On street sweepings at roadside, half-buried, Golden Gate Park, San Francisco, N. L. Gardner *188*, Feb. 29, 1904," UC, PARATYPE of *Daleomyces gardneri* Setchell.

The cupulate (and probably more common) form of the species should bear the name:

PEZIZA (subgen. GALACTINIA) PROTEANA (Boud.) Seaver forma **proteana**
= *Aleuria* (*Galactinia*) *proteana* Boud. [var. *proteana*], Bull. Soc. Myc. Fr. **15**: 50. 1899. (!!)
= *Galactinia proteana* (Boud.) Sacc. & Syd. *in* Sacc., Syll. Fung. **16**: 709. 1902.
= *Peziza proteana* (Boud.) Seaver, Mycologia **9**: 1. 1917.

TYPE SPECIMEN EXAMINED:

France: "Galactinia proteana Boud. in carbonariis. Forêt de Carnelle. 1886," PC-B, HOLOTYPE of *Aleuria proteana* Boud.

II. A SECOND SPECIES OF DALEOMYCES

Only one additional species appears to have been described in the genus *Daleomyces*, namely *D. shearii* Gilkey (**9**). I have had the opportunity of examining the type specimen, and find that it is morphologically very similar to *Peziza proteana* f. *sparassoides*. Miss Gilkey's placement of this species in *Daleomyces* was wholly logical, for

if *P. proteana* f. *sparassoides* (= *Daleomyces gardneri*) is a member of a special genus of the Tuberales, her species is certainly congeneric!

My investigation of the type specimen of *D. shearii* shows that it, also, has evidently operculate asci which blue in iodine, warted spores (well illustrated by Miss Gilkey, **9**), and a compound ascocarp. The ascocarp is small (3 cm diam.) in the only known specimen, scarcely larger than the smallest known specimens of *P. proteana* f. *sparassoides*. Like the latter it belongs to the genus *Peziza*, and to the same subgenus, *Galactinia*. The spores are much larger and broader (subspherical) than those of *P. proteana*, usually have only a single guttule, and are slightly brown (but the brown color may be due to fixation and embedding of the specimen). They recall the spores of *Galactinia tosta* Boud. (**5**: pl. 285), but my examination of authentic (type?) specimens of the latter indicates spore and tissue differences. The subspherical, perhaps brownish, spores recall some species of *Plicaria* sensu Boudier, but do not match any species known to me. Since I know of no cupulate species of *Peziza* or of "*Plicaria*" that has similar spore and tissue characters, I am accepting it as a good species: **Peziza** (subgen. *Galactinia*) **shearii** (Gilkey) Korf, comb. nov. (basionym: *Daleomyces shearii* Gilkey, Oreg. St. Monogr., Stud. Bot. **1**: 26. 1939).

In Miss Gilkey's (**10**) most recent treatment, she has excluded "*Daleomyces gardneri*" from the Tuberales, and has added a note to indicate that this disposition leaves the placement of *D. shearii* in doubt. On the basis of my investigations, both species belong in *Peziza* despite their bizarre development of compound ascocarps.

III. Sparassoid forms of other operculate Discomycetes

Two sparassoid species, both apparently referable to *Peziza* subgen. *Galactinia*, were described by Hennings (**11**) from Java. *P. sparassiformis* (P. Henn. *in* Warb.) Sacc. & Syd. *in* Sacc. is quite distinct from *P. proteana* f. *sparassoides* and from *P. shearii*, to judge from the original description and plate. *P. tjibodensis* (P. Henn. *in* Warb.) Sacc. & Syd. *in* Sacc. would appear from the description to be a sparassoid form, though less highly developed, of yet another species of *Peziza*. Unfortunately I have been unable to locate the type specimen of either species for study.

In the type subgenus of *Peziza*, the saccate form of the type species, *P. vesiculosa* Sow. ex Hook. f. *saccata* Fr., may be considered as representing a less highly developed condition wholly analogous to the compound ascocarp of the sparassoid forms mentioned above. Boudier's

(**5**: pl. 258) excellent plate should be consulted and compared with illustrations of *P. proteana* f. *sparassoides*.

The development of sparassoid and saccate fruitbodies is not restricted to the genus *Peziza*, however. *Disciotis venosa* (Pers. ex Pers., Boud. var. *reticulata* (Grev.) Boud. is at least a saccate if not quite sparassoid form, and has also been beautifully illustrated by Boudier (**5**: pl. 255). *Discina convoluta* Seaver would appear from the description and illustration (**22**) to be merely a saccate form of *Discina leucoxantha* Bres. I have noted in the field the development of essentially similar saccate fruitbodies of *Discina perlata* (Fr.) Fr. (= *D. ancilis* sensu Seaver). On the basis of field observations I concur with the opinion of Boudier (**3**, **4**), who has pointed out that the saccate condition of the fertile cap of *Ptychoverpa bohemica* (Kromb.) Boud. appears with maturity, and may be considered as the "normal" development in that species.

The fruitbody of *Underwoodia columnaris* Peck does not, however, represent a sparassoid development as in the preceding species. The elongate fruitbody possesses vertically elongated chambers as illustrated by Seaver (**22**) and Nusslé (**18**). The hymenium is wholly restricted to an outer layer, and there is no evidence of a compound ascocarp in any of the specimens I have seen. The major portion of the ascocarp in this species consists of a fluted and chambered sterile tissue analogous to the stipe tissue in a number of species of *Helvella* (cfr. **5**: pl. 227–230) and *Neogyromitra* (**5**: pl. 221), over the surface of which is borne the hymenial layer. The *Underwoodia*-type of development is not at all analogous to the saccate-sparassoid type of development.

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NEW GENERA OF FUNGI. VII [1]

ROLF SINGER

The present paper deals with certain small groups and isolated species, generally analogous to the Trichomolas and Clitocybes (tribus *Clitocybeae*) but differing from them by amyloid spores. This problem can be approached from the theoretical as well as from the practical side. The former approach is perhaps the more attractive one from the point of view of a general interest, although it is bound to lead us nowhere in particular, since it involves such *a priori* unanswerable questions as the origin of the amyloid reaction of the spore wall from a phylogenetic standpoint, and its importance, in principle, for generic and specific systematics in the Basidiomycetes. Although there is not one single mycologist who gives the character an absolute validity by stating that species with different spore chemistry cannot belong to the same genus, there is still a relatively wide range of opinions as to the extent of validity attributable to this character. Neuhoff,[2] in a somewhat anachronistic paper takes the most radical view. He denies, against all the evidence accumulated by almost an entire generation of modern taxonomists, any importance of amyloidity in spore or hyphal walls for the systematics of the Agaricales. A. H. Smith and R. W. G. Dennis as well as Kühner & Romagnesi deny the generic importance of the character in certain groups (like *Mycena*) while maintaining it as a generic character in other cases. The author of the present paper has repeatedly made use of the character where it seemed helpful, recognizing at the same time that it is a sectional or specific character in other cases (as in *Amanita, Cystoderma,* certain Mycenae). I am not aware of any definite hypothesis on the phylogenetic level, which prefers to consider amyloid-spored species on principle more recent or more primitive, or which considers the character as such, basically either primitive or a recent development. Under these circumstances, it is obvious enough that we have to deal with each taxonomic problem on its own merits.

[1] Earlier parts of this series have been published as follows: I. Mycologia **36**: 358–368. 1944. II. Lloydia **8**: 139–144. 1945. III. Mycologia **39**: 77–89. 1947. IV. Mycologia **40**: 262–264. 1948. V. Mycologia **43**: 598–604. 1951. VI. [as V.] Lilloa **23**: 255–258. [1950] 1951.

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We can only hope that when all these problems have been solved more or less satisfactorily, the data thus obtained may lend themselves to certain conclusions of a more general nature.

The solution of taxonomic problems becomes easier the more facts are known about the representatives of a group, viz., the more characters of the known species have been studied, and the more representatives of the groups involved are fully described. I have, in previous papers, repeatedly indicated that research on tropical and generally "exotic" floras plays an important rôle far beyond the program of getting acquainted with the plant life of the entire globe, insofar as it leads to the discovery of species which may throw a new and often revealing light on otherwise difficult taxonomic problems. This method has been applied successfully for the solution of the question regarding the status of the genus *Poromycena* Van Overeem. It was found that an abrupt hiatus between the section *Purae* of *Mycena* and the genus *Poromycena* does not exist, since certain forms of *Mycena* aff. *pura* with strongly poroid hymenophore exist in the American tropics (see *Dictyoploca holoporphyra* (Berk. & Curt.) Dennis in Dennis, Trans. Brit. Myc. Soc. **34**: 475. 1951; *Mycena pura* (aff.) R. Singer & A. P. Digilio, Lilloa **25**: 237–238. [1951] 1952; cf. also Singer, Mycologia **45**: 886, footnote 6. 1953) which approach *Poromycena violacella* to such a degree that the only difference remains in the reaction of the spore wall. I also wish to recall the solution of the problem regarding the position of *Fayodia* subgenus *Clitocybula* which became possible only through the studies made on Patagonian representatives of that group (see Singer, Mycologia **45**: 886. 1953 and Sydowia **8**: 110–111. 1954). Other examples are the analyses of the genera *Thaxterogaster* (Singer, Mycologia **43**: 215–228. 1951) and *Brauniella* (Singer, Lilloa **26**: 102–108. 1953) as well as several details of the classification of the boletes which could not be solved by examining merely the European representatives of that group.

In the case of the amyloid-spored Tricholomas and Clitocybes, we have likewise been able to study some typically "exotic" groups of species which, in my opinion, throw a new light on the entire problem. First of all, we have in the area of the *Nothofagus* woods of southern South America an interesting genus, *Porpoloma* Sing., which, in spite of its external similarity with species of *Tricholoma* (to the point that each of the three representatives may be mistaken for one common species of *Tricholoma*, a feature expressed in the specific names proposed for them, see Sydowia **6**: 198–201. 1952), differs in several, not merely one, characters of importance so far as structural and microchemical

characters are concerned. Kühner, discussing the problem of the amylosporous Tricholomas in a recent paper, remarks that all the species of tricholomatoid habit and with amyloid spores have clamp connections with the one exception of the genus *Melanoleuca,* and that they are small groups of species or isolated species with a strong hiatus between each of them; these two observations lead him to observe that this leads to the supposition of a rather ancient origin of their various forms and of the entire group of amylosporous tricholomatoid agarics. This reasoning seems to me quite sound. It rejects the obviously fallacious idea of an extension of the genus *Leucopaxillus* to receive all smooth-amyloid-spored Tricholomataceae (with the exception of the mycenoid groups) or of a combination of *Melanoleuca* and *Leucopaxillus* (as proposed by Métrod), and explains the isolatedness of the groups in question by their origin "at the phylogenetic level of the Clitocybes." While we can agree with this point of view, we shall now see how exotic cryptogamy enters the problem. Kühner starts his discussion with the description of an interesting species which he determines as *Tricholoma pes-caprae* (Fr.). Comparing this description with the diagnosis of the genus *Porpoloma,* one will immediately find full agreement in all essential characters, including the similarity of this species with known species of *Tricholoma* proper; the only point of divergence may be in the ecology of the species involved; while there is reason to believe (although experimental data are still wanting) that the Patagonian species are mycorrhizal with *Nothofagus,* Kühner indicates that *T. pes-caprae* occurs exclusively in montane meadows (for example at 1000–1300 m altitude, with *Melanoleuca grammopodia*) which would indicate that we have here a species without mycorrhizal relations, and consequently, if anything, still more primitive than the *Nothofagus*-symbionts, a conclusion which is also indicated by the fact that it belongs to a group mainly inhabiting the subantarctic regions where—as we have shown in previous papers—most floral elements (so far as fungi are concerned) belong to relatively ancient floras, either the elements of a flora with now subantarctic-subarctic geographical disjunction (cf. Botanical Review **20**: 459. 1954), or members of the old austral-antarctic flora (cf. Sydowia **9**: 367–431. 1955), while only a small minority of isolated species of various genera can be assumed to have migrated in a north-south direction via the Andes. This floral analysis would, in turn, permit corroboration of Kühner's reasoning regarding the amylosporous tricholomatoid agarics. Since, on the other hand, the genus *Tricholoma* as a whole (although in its present limits also containing some rather ancient elements), may be considered as about as recent as

the typical Clitocybes, we cannot classify *Tricholoma pes-caprae* with the other Tricholomas, but have to transfer it to *Porpoloma* as a special subgenus:

PORPOLOMA Sing.

Subgenus **Porpoloma.** Context odorless; mycelium apparently mycorrhizal; species of the Southern Hemisphere: *P. sejunctum, P. portentosum, P. terreum.*

Subgenus **Pes-caprae** Sing. subgen. nov. Carne farinolente; mycelio apparenter haud mycorhizogeno; species borealis. Species TYPICA: *P. pes-caprae* (Fr.) Sing. comb. nov. (*Agaricus pes caprae* Fr. Epicr. 45. 1836).

Another group of species, obviously similar to those mentioned above, has been observed in the American tropics. It is represented by *Dennisiomyces griseus* (Dennis) Sing. and *D. glabrescentipes* Sing., the first from Trinidad, the second from Brazil.

The genus *Dennisiomyces* was described as having partly ascendant or erect-depressed terminal cells of the epicuticular superficial hyphae which are similar in shape to the cheilocystidia, which are numerous and make the edge of the lamellae heteromorphous. Broader and more conspicuous, very numerous, cystidia are also found on the sides of the lamellae. *D. glabrescentipes* is probably terrigenous; *D. griseus* grows on humus. Both have hyphae with clamp connections and smooth amyloid, ellipsoid spores. Their habit is definitely tricholomatoid.

These species are not closely related to any known agaric. Comparing them with other amyloid-spored Tricholomataceae, one might think of *Porpoloma, Hydropus,* and *Heimiomyces,* considering the *Dennisiomyces* species tentatively as pleurocystidiate representatives of those genera whose diagnosis would have to be emended to accommodate the two species. However, they differ from *Porpoloma* not merely in the presence of pleurocystidia, but in the structure of the epicutis, aside from the fact that there does not appear to be any similarity in habitat and area. As for *Hydropus,* there is also a definite difference between the known species of that genus and the two species mentioned above as far as the structure of the epicutis of the pileus is concerned. The ascendant or erect cells of the latter are always typically broad and rather voluminous in *Hydropus,* and the habit of the species is mycenoid-collybioid-omphaliod rather than tricholomatoid. *Heimiomyces* might be the genus one is most likely to arrive at when attempting to determine the two neotropical species with the keys available for the identification of agaric genera. Nevertheless, the appearance of true *Heimiomyces*

species is that of the Collybias,[3] and not of the Tricholomas. Aside from that, pleurocystidia of the type found in *Dennisiomyces* are absent in all species of *Heimiomyces*. All species of *Heimiomyces* are lignicolous and white to bright colored, while *Dennisiomyces* is not definitely lignicolous, and possesses brown to fuscous pigmentation.

The two species of *Dennisiomyces* were described by Dennis (Trans. Brit. Mycol. Soc. **34**: 460. 1951) and Singer (Type Studies on Basidiomycetes—VIII, Sydowia **9**: 395–396. 1955) and by Singer (Anais da Sociedade de Biologia de Pernambuco **13**: 225. 1955).

The third species entering this problem is *Collybia cavipes* Pat. & Gaill. as interpreted by Dennis, Trans. Brit. Mycol. Soc. **34**: 440. 1951, and illustrated on his pl. 21, f. 1. This, however, has a more definitely collybioid-omphalioid habit, grows on rotten logs, and reminds one, in its external appearance, rather of the genera *Gerronema* and *Clitocybula*, the former differing in inamyloid spores and absence of pleurocystidia; the latter is also mainly acystidiate, but *Clitocybula abundans* has rather voluminous and constant cheilocystidia, and an emendation of the genus for the purpose of accommodating *C. cavipes* does not seem unnatural; on the other hand, the spores which are less short than ordinary, and the structure of the epicutis, would lead one rather to the genus *Hydropus* where cystidiate species are not characteristic but may be expected because some of the species entering that genus have occasional pleurocystidia similar to the cheilocystidia and away from the edge of the lamellae, as we have observed in *H. marginatus* var. *rugosodiscus* from New Hampshire, coll. Linder and Singer (FH). Cystidiate species once admitted, there cannot be much objection to inserting *C. cavipes* in the genus *Hydropus*, and for that reason as well as due to the absence of type material in our herbarium, we refrain from insisting on the affinity of *C. cavipes* and the *Dennisiomyces* species indicated above, and merely limit ourselves to calling attention to the existence of another amylosporus cystidiate species in the American tropics.

[3] A. H. Smith has proposed to combine *Heimiomyces* with *Xeromphalina* because of the similarity of the covering of the stipe and the colors. However, here again, a solution can be found only if all species known rather than those of a local flora are considered. *H. pruinatipes* and *H. brunneipes* do not fit into Smith's combined genus. Moreover, anyone who keeps *Collybia* and *Omphalina* or *Clitocybe* apart because of the macroscopical characters should likewise separate *Heimiomyces* inasmuch as *Xeromphalina* is a predominantly temperate northern genus, and *Heimiomyces* a predominantly tropical-subtropical genus. If among Smith's species of *Xeromphalina* sensu lato only *X. tenuipes* is removed to *Heimiomyces*, the separation of the two certainly closely related genera becomes quite easy and natural, the stipe being evenly pruinate to velvety in *Heimiomyces*.

A third group of species, obviously independent of the ones just treated, consists of a single European representative known to me, and one more neotropical species, described by Dennis as *Tricholoma atrobrunneum* Dennis, recently transferred by me to *Dermoloma* (Lange) Sing.

The genus *Dermoloma* (Lange) Sing. was until recently recognized only on a temporary basis since its relations to the Tricholomas with cellular epicutis were somewhat doubtful, inasmuch as it was based on a single species. The discovery of a second species in the tropics, with essentially identical decisive characters shows that the genus is not merely representative of an extreme ramification of the Tricholomas but can be considered a well defined small group of species of the amylosporous tricholomatoid agarics. It is significant that the two species known at present belong to two extremely different floras and are widely separated geographically. This situation constitutes a phenomenon parallel with that observed in the case of the genus *Porpoloma*, and underscores Kühner's conclusion that these amylosporous groups are not of recent origin. The tropical species, as the temperate one, is apparently non-mycorrhizal, clamp-bearing, smooth-spored, and covered by a hymeniform layer of spherocysts on the pileus.

DERMOLOMA (Lange) Sing., Lilloa **22**: 250. 1949 (1951) "ad int." ex Sing.

Tricholoma subgenus *Dermoloma* Lange, Dansk Bot. Ark. **8**: 12. 1933.

Species TYPICA: *D. cuneifolium* (Fr.) Gill. sensu Josserand, i.e., sensu Lange (*Tricholoma cuneifolium* sensu Joss.; Lange, Dansk. Bot. Ark. **8**: 12); other species: *D. atrobrunneum* (Dennis) Sing. (= *Tricholoma atrobrunneum* Dennis, Trans. Brit. Myc. Soc. **34**: 476. 1951).

I do not wish to enter the question of the taxonomic position of *Tricholoma elytroides* (Fr.) sensu Romagnesi since I have not studied personally the European species. I have seen material of the American species considered synonymous by Romagnesi, viz. *Tricholoma umbrosum* A. H. Smith & Walter, but the characters of that species did not seem to coincide fully with the description of the European species, and my material is not sufficient to corroborate or contradict the conclusion that the latter is merely an extreme form of the former. In itself, the possibility of a necessity of emending the genus *Dermoloma* so as to accommodate, aside from *D. cuneifolium* and *D. atrobrunneum*, also forms with an indistinct or reduced epithelium or hymeniform layer

such as *T. elytroides* cannot be rejected *a priori*. Similar cases are known to present themselves, e.g. in *Calocybe*, in *Tricholoma*, and, as recent research on tropical Clitocybes shows, also in the genus *Clitocybe*, since we have been able to study a species with all characteristics of a typical *Clitocybe* but with an epicutis made up in the same manner as the epicutis of *Dermoloma*.

The data given here concerning the genera *Porpoloma*, *Dennisiomyces*, *Dermoloma* suggest that the groups assembled in my genus *Cantharellula* in the wider sense should not be kept in *Cantharellula* as subgenera but rather be treated on an equal footing with the genera just mentioned. Only regarding the subgenus *Pseudotricholoma* Sing. it seems to be wiser to wait for its eventual clarification so far as its relations to *Tricholoma elytroides* are concerned (see above). This leaves for immediate disposal the subgenera *Pseudoarmillariella*, *Pseudoomphalina*, and *Pseudoclitocybe* which I believe now to be worthy of generic status:

Pseudoarmillariella (Sing.) Sing. stat. nov. (genus)

Synonym: *Cantharellula* subgenus *Pseudoarmillariella* Sing., Sydowia **2**: 29. 1948. TYPE: *P. ectypoides* (Peck) Sing. comb. nov. (= *Cantharellula ectypoides* (Peck) Sing., Lloydia **5**: 120. 1942).

Pseudoomphalina (Sing.) Sing. stat. nov. (genus)

Synonym: *Cantharellula* subgenus *Pseudoomphalina* Sing., Sydowia **2**: 30. 1948. TYPE: **P. kalchbrenneri** (Bres.) Sing. comb. nov. = *Omphalia kalchbrenneri* Bres., Fung. Trid. **1**: 32. 1883.

Pseudoclitocybe (Sing.) Sing. stat. nov. (genus)

Synonym: *Cantharellula* subgenus *Pseudoclitocybe* Sing., Ann. Mycol. **41**: 64. 1943. TYPE: **P. cyathiformis** (Bull. ex Fr.) Sing. comb. nov. (= *Cantharellula cyathiformis* (Bull. ex Fr.) Sing., Rev. de Mycologie **1**: 281. 1936).

In both *Pseudoomphalina* and *Pseudoclitocybe* some additional species are enumerated in Lilloa **22**: 238–239. [1949] 1951.

In order to summarize the conclusions presented in the present paper, I add a key to the various groups treated (amylosporous tricholomatoid and clitocyboid species of agarics, not including the *Binnulariceae*, viz. genera *Armillaria* and *Catathelasma*):

A. Hyphae of the carpophore with clamp connections; spores smooth
  B. Cheilocystidia present and numerous, well differentiated
    C. Pleurocystidia also present, numerous and well differentiated
      D. Habit tricholomatoid, often humicolous; lamellae sinuate; context fleshy; spores ellipsoid; hymenophoral trama inamyloid; tropical species.....*Dennisiomyces*
      D. Not combining these characters, cf. *Lentinellus*, Myceninae, etc.
    C. Pleurocystidia absent, or extremely rare near edge, or very inconspicuous; extratropical species
      D. Habit tricholomatoid, humicolous; lamellae sinuate; context fleshy; spores ellipsoid.....*Porpoloma*
      D. Not combining all the characters indicated
        E. Pileus usually more or less innately radially fibrillose; spores often rather short; color brownish gray, whitish gray, grayish fuscous, rarely melleous; lignicolous, temperate species..*Clitocybula*
        E. Not combining all these characters, cf. *Pseudoomphalina*
  B. Cheilocystidia absent or inconspicuous, inconstant, and scattered
    F. Epicutis consisting of spherocysts which form a hymeniform layer
      G. Habit tricholomatoid, lamellae usually sinuate, context fleshy, habitat on humus, not mycorrhizal, tropical and extratropical.....*Dermoloma*
      G. Not combining these characters, cf.....Myceninae
    F. Epicutis different
      H. Habit clitocyboid or omphalioid
        I. Pileus opaque and subvelutinous; lamellae characteristically repeatedly forked; context soft and with a tendency to redden on exposure; spores elongated; bryophilous.....*Cantharellula*
        I. Not combining these characters
          J. Cespitose, wood-inhabiting species, habit of *Armillariella chrysophylla*; lamellae often more or less forked; spores ellipsoid, about twice as long as broad.....*Pseudoarmillariella*
          J. Not combining these characters
            K. Gigantic, fleshy species with initially involute, slightly subtomentose margin.....*Leucopaxillus*
            K. Small to medium sized species, moist to hygrophanous, or with radial innate fibrillosity, sometimes carbonicolous or lignicolous; margin of pileus never involute or subtomentose
              L. Pileus dull colored (gray, umber, fuscous, fuliginous, whitish cinereous etc.), mostly distinctly innately radially fibrillose; spores often relatively short; lignicolous.....*Clitocybula*
              L. Pileus flesh-buff, ochraceous alutaceous, clay color, orange-cinnamon, pale orange, etc., not innately radially fibrillose, but moist to hydrophanous; spores ellipsoid; habitat on earth or charcoal.....*Pseudoomphalina*
      H. Habit tricholomatoid
        M. Gigantic light-colored fleshy species with initially involute and subtomentose but not spinulose margin.....*Leucopaxillus*
        M. Not combining these characters

N. Medium sized carpophores with rather dusky dull coloration (but sometimes with a tendency to become yellow or reddish inside), not quite glabrous, humicolous or on roots; odor strong (flour, cucumbers, sweetish)

O. Pigment vacuolar (intracellular); margin of pileus involute and spinulose; spores small and ellipsoid, cf. ..... *Leucopaxillus spinulosus*

O. Pigment membranal; spores elongated, cf. *Tricholoma elytroides* sensu Romagnesi & LeGal et al.?, *T. umbrosum* Sm. & Walters (*Cantharellula*, subgen. *Pseudotricholoma*)

N. Not combining these characters (see A below)

A. Hyphae of the carpophore clampless, or spores not smooth. See *Melanoleuca, Lentinellus, Leucopaxillus* subgenus *Leucopaxillus, Pseudoclitocybe* (= *Cantharellula* subgenus *Pseudoclitocybe*), and cf. Myceninae (large individuals)

INSTITUTO MIGUEL LILLO,
TUCUMÁN, R. ARGENTINA

TROPICAL FUNGI IMPERFECTI [1]

EVERETT F. MORRIS

(WITH 6 FIGURES)

This paper consists of a list of hyphomycetes which were isolated from fragments of palm leaves, angiospermous wood and a termite nest collected in the Republic of Panamá and the Panamá Canal Zone. The materials were collected by G. W. Martin and A. L. Welden in July and August, 1952, under a National Science Foundation Grant.

The materials were wrapped in newspaper obtained in Panamá and then kept enclosed in manila envelopes until opened for this study. Portions of the materials were removed from their containers by use of sterile forceps and were placed into sterilized petri dishes. The materials were moistened with carbon treated distilled water repeatedly throughout the course of study.

In order to study development of conidiophores and conidia, certain of the isolates were grown on micro-culture slides. Weak potato-dextrose agar was used as the medium in the micro-cultures. The micro-culture slide described by Shoemaker (**9**) was used.

Abbreviations of the collecting areas are as follows: Barro Colorado Island, BC.; Chiriquí Province, Chir.; Fort Sherman area, FS.

PAECILOMYCES VARIOTI Bainier, Bul. Soc. Mycol. France **23**: *26*, Pl. 8. 1907.[2]

BC. Cultured from palm leaf collected 23 June 1952; put in moist chamber 5 March 1954. GWM *8763*. Cultured from palm leaf collected 28 June 1952; put in moist chamber 1 Nov. 1954. GWM *8765*.

GLIOCLADIUM PENICILLOIDES Corda, Icon. Fung. **4**: 31, f. 92. 1840.[2]

BC. Cultured from old termite nest collected 3 July 1952; put in moist chamber 24 Feb. 1954. GWM *8764*.

[1] Excerpt from thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the State University of Iowa.

[2] These species were reported from same localities by Farrow, Mycologia **56**: 632-651. 1954.

TRICHOTHECIUM ROSEUM Link ex Fries, Syst. Mycol. **3**: 427. 1829.

BC. Cultured from palm leaf collected 18 July 1952; put in moist chamber 12 March 1953. GWM *8766*. Cultured from angiospermous wood collected Aug. 1952; put in moist chamber Sept. 1952. GWM *8767*.

DIPLOSPORIUM ALBUM Bonorden, Handb. allgem. Mykol. 99, f. 108. 1851.

BC. Cultured from angiospermous wood collected July 1952; put in moist chamber autumn 1952. GWM *8768*.

PERICONIA IGNIARIA Mason & M. B. Ellis, C.M.I., Mycol. Papers **56**: 104, f. 30, 31. 1953.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 1 Nov. 1954. GWM *8769*.

A red or wine-colored pigment is formed in culture and colors the medium throughout the growing area. A reddish-buff pigment has been noted in certain isolates of another species, *P. byssoides* (**8**). Other than this, I have found no references to pigment formation in the genus. The fungus grows very slowly in culture and does not form stromata. This agrees with the experience of Mason and Ellis, who did not find formation of stromata in culture. The isolate studied is somewhat intermediate between *Periconia circinata* and *P. igniaria* as described by Mason and Ellis. It resembles *P. circinata* in forming chlamydospores and in occasionally producing circinate conidiophores (**7**). The long spines of the conidia and pigment formation in culture have not been reported in *P. circinata*. It seems desirable to give more importance to the spore and pigment characters and refer this fungus to *P. igniaria*.

?PERICONIA sp.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 5 March 1954. GWM *8770*.

The conidia do not form a head at the apex of the conidiophore, but are borne on short branches near the apex and lower down on the conidiophore. The conidia are produced singly at the tips of the short branches or in acropetalous chains. Occasionally, a branch grows past a conidium and produces a new conidium at its tip.

The only fungus of which I have been able to find a description which resembles this isolate is *Periconia cambrensis* Mason & M. B. Ellis (**8**). The spores of *P. cambrensis* are smaller than those of the present species.

STACHYBOTRYS ATRA Corda, Icon. Fung. **1**: 21, f. 278B. 1837.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 1 Nov. 1954. GWM *8792*.

PHIALOPHORA VERRUCOSA Medlar, Mycologia **7**: 203, f. 1. 1915.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 1 Nov. 1954. GWM *8771*.

GONYTRICHUM MACROCLADUM (Sacc.) Hughes, Trans. Brit. Mycol. Soc. **34**: 565, f. 3. 1951.[2]

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 5 March 1954. GWM *8772*. Cultured from angiospermous wood collected 18 Aug. 1952; put in moist chamber 20 Sept. 1952. GWM *8740*.

CORDANA PAUCISEPTATA Preuss, Linnaea **24**: 129. 1851.

BC. Cultured from angiospermous wood collected 11 July 1952; put in moist chamber 24 Feb. 1954. GWM *8773*.

CLADOTRICHUM sp.

BC. Cultured from angiospermous wood collected 24 July 1952; put in moist chamber 18 Sept. 1953. GWM *8793*.

CLADOSPORIUM HERBARUM (Pers.) Link ex Fr. Syst. Mycol. **3**: 370. 1829.[2, 3]

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 1 Nov. 1954. GWM *8774*.

TORULA HERBARUM Link ex Fries, Syst. Mycol. **3**: 501. 1829.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 5 March 1954. GWM *8775*.

CURVULARIA GENICULATA (Tracy & Earle) Boedijn, Bull. Jard. Buitenzorg **13**: 129, f. 3(3), 4(1). 1933.[2]

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 5 March 1954. GWM *8776*.

[3] These species were reported from "Panama" by White *et al*, Mycologia **40**: 34–84. 1940. Most of them were presumably from the Canal Zone area, including Barro Colorado Island.

FS. Cultured from leaf of *Cocos nucifera* collected 26 Aug. 1952; put in moist chamber in autumn of 1953. GWM *8778*.

Chir. Cultured from angiospermous wood collected 2 Aug. 1952; put in moist chamber in autumn of 1953. GWM *8779*.

CURVULARIA LUNATA (Wakker) Boedijn, Bull. Jard. Buitenzorg **13**: 127, f. 3(2), 3(12), 4(3). 1933.[2, 3]

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 5 March 1954. GWM *8777*.

SPONDYLOCLADIUM XYLOGENUM A. L. Smith, Trans. Brit. Mycol. Soc. **3**: 37, Pl. 1, f. 5, a, b. 1908.

BC. Developed on angiospermous wood collected 11 July 1952; put in moist chamber 24 Feb. 1954. GWM *8780*. I did not succeed in getting this fungus in culture.

SPONDYLOCLADIUM OBOVATUM (Cooke & Ellis) Hughes, Can. Jour. Bot. **31**: 634, f. 80. 1953.

BC. Developed on angiospermous wood collected 19 July 1952; put in moist chamber 24 Feb. 1954. GWM *8781*. I did not succeed in getting this fungus in culture.

The conidia are produced solitarily on the apices of the conidiophores and laterally in irregular whorls as in *S. xylogenum*. Conidiophore proliferation, as in *S. xylogenum*, was not found, but lateral production of conidia was more evident.

SPONDYLOCLADIUM sp.

BC. Developed on angiospermous wood collected 3 July 1952; put in moist chamber 24 Feb. 1954. GWM *8782*. I did not succeed in getting this fungus in culture.

This fungus appears quite similar to the two previously mentioned species of *Spondylocladium*, but no lateral conidia were observed. The conidia differ in that the terminal cell is much larger proportionally to the lower cells, in larger size, and in having short stalks. The fungus differs from *Brachysporium*, as emended by Mason and Hughes (**5**), in producing conidia singly in the apical region. I have found no description of a *Spondylocladium* which agrees with this species; it is possibly undescribed.

STEMPHYLIUM CONSORTIALE (Thüm.) Groves & Skolko, Can. Jour. Res. **22C**: 196, Pl. 1, f. 7; Pl. 5. 1944.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 1 Nov. 1954. GWM *8783*.

This apparently common species has been poorly understood. Groves and Skolko (**3**), in proposing the combination *S. consortiale*, figured and described the species in 1944. In their paper, no mention was made of the occurrence of conidia in chains. In 1953, Groves and Hughes (**6**) proposed the combination *Alternaria consortiale* and stated, "chains of conidia are not infrequent." This does not seem to be a good characteristic for the separation of *Stemphylium* and *Alternaria*. Furthermore, chains have been reported in *Stemphylium*, as is evident by the statement of Skolko and Groves ". . . in contrast to the *Alternaria* chain where the distal spore is the youngest, in the *Stemphylium* chain the distal spore is the oldest." *Stemphylium* spores are oblong to packet-shaped whereas those of *Alternaria* are obclavate. In the isolate here reported, I have found conidial chains to be of very infrequent occurrence while the *Stemphylium*-type spore is most evident. The obvious difficulty seems to be the decision as to which characteristic is the more fundamental. I believe the conidial shape to be the more useful in the separation of the two genera and have chosen to retain the name *Stemphylium consortiale* for the species listed here.

SPORODESMIUM BAKERI Sydow, Annales Mycologici **12**: 204. 1914.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 1 Nov. 1954. GWM *8784*.

GRAPHIUM RIGIDUM (Pers. ex Fries) Saccardo, Sylloge Fungorum **4**: 610. 1886.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 5 March 1954. GWM *8785*. Cultured from *Apeiba* fruit collected Aug. 1952; put in moist chamber Nov. 1952. GWM *8743*. It also occurs very commonly on angiosperm wood in Panamá.

STILBELLA sp.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 24 March 1954. GWM *8786*.

MYROTHECIUM RORIDUM Tode ex Fries, Syst. Mycol. **3**: 217. 1829.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 5 March 1954. GWM *8788*.

FUSARIUM OXYSPORUM Schlecht. ex Fries, Syst. Mycol. **3**: 471. 1829.

Chir. Cultured from angiospermous wood collected 2 Aug. 1952; put in moist chamber in autumn of 1953. GWM *8787*.

PESTALOTIA DICHAETA Spegazzini, Anal. Mus. Nas. Buenos Aires, Ser. III. **13**: 411. 1910.

BC. Cultured from palm leaf collected 28 June 1952; put in moist chamber 27 Feb. 1954. GWM *8789*.

If recently proposed changes were followed, this fungus would be listed as *Pestalotiopsis dichaeta* (Speg.) Steyaert. In 1949, Steyaert (**10**) proposed two new segregates from the genus *Pestalotia*. All 4-celled species of *Pestalotia* were placed in the new genus *Truncatella*, making a total of 5 species in the new genus. The second segregate, *Pestalotiopsis*, was proposed to include 40 species of *Pestalotia* which have 5 cells, in addition to 11 newly described species (**11**, **12**). Steyaert proposed an emended description of *Pestalotia* and for the genus retained 1 species, *Pestalotia pezizoides*, which has 6 cells. In addition to the septation of the spores, Steyaert justified the erection of the two new segregates on the basis of their having different types of fruiting bodies. He maintained that the fruiting structure of *P. pezizoides* was a conceptacle instead of an acervulus as in all of the other species of *Pestalotia*.

I do not believe that a slightly different fruiting structure and septation of spores, in a genus where septation is not constant, justify these changes. Furthermore, in culture the fruiting body of this fungus is a sporodochium. I prefer to designate this species as *Pestalotia dichaeta* Speg.

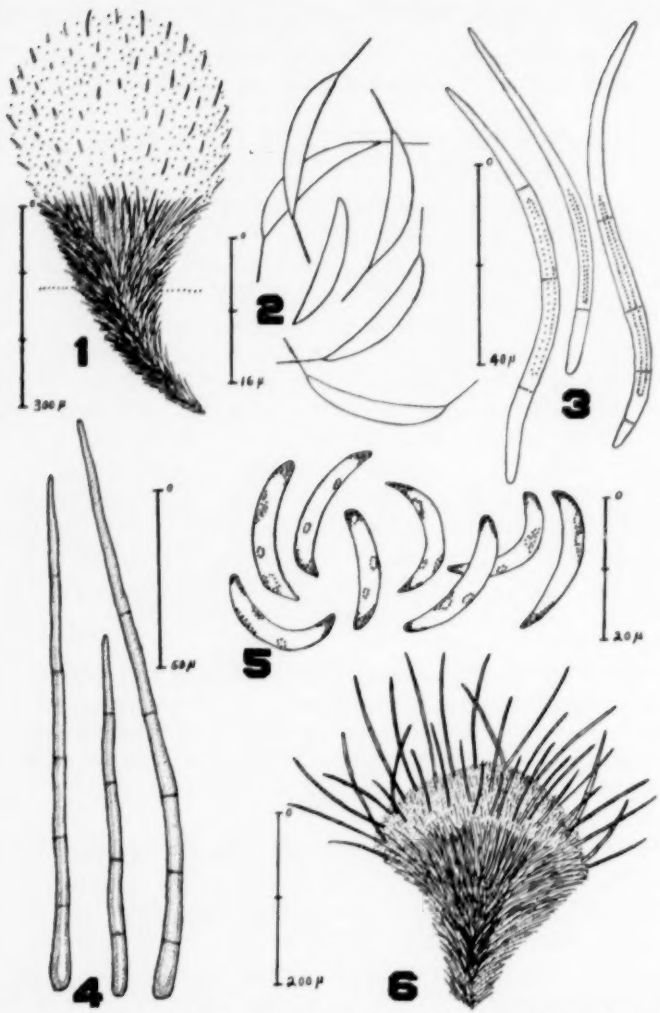
PESTALOTIA DISSEMINATA Thümen, Inst. Rev. Sci. Coimbra **28**: 501. 1880.

FS. Cultured from palm leaf collected Aug. 1952; put in moist chamber autumn 1952. GWM *8790*.

Steyaert (**12**) proposed the new combination *Pestalotiopsis disseminata* (Thüm.) Steyaert for this species, but I have chosen to designate it as indicated.

Schizotrichella gen. nov.

Sporodochiis subglobosis, superficialibus, nigris; setis multis, simplicibus, septatis, nigris, acutis; conidiophoris obclavatis, apice ramoso; conidiis lunatis, continuis, hyalinis.



FIGS. 1–3. *Neottiosporella radicata*. 1. Habit of sporodochium. 2. Conidia. 3. Setae. FIGS. 4–6. *Schizotrichella lunata*. 4. Setae. 5. Conidia. 6. Habit of sporodochium.

Sporodochia subglobose, superficial, black; setae many, simple, septate, black, pointed; conidiophores obclavate, branching at the apex; conidia lunate, unseptate, hyaline.

Type species: *S. lunata.*

Schizotrichella lunata sp. nov. FIGS. 4–6.

Sporodochiis gregariis, 140–350 μ diam., sessilibus vel substipitatibus; setis nigris septatis, 95–225 × 3.5–7 μ, in marginibus et irregulariter dispersis super sporodochia; conidiophoris dense aggregatis, totas fructificationes obducentibus, ramosis cum ultimis ramis compactum stratum facientibus; conidiis abundanter in serie productis, aggregatis, hyalinis, guttulatis, continuis, lunatis, 17.5–21 × 2.5–3 μ.

Sporodochia gregarious, 140–350 μ in diameter, sessile, or on a short stalk, with dark septate setae, 95–225 × 3.5–7 μ, at the margin and scattered irregularly over the sporodochium; conidiophores densely crowded, covering the entire fructification, branched with the ultimate branches forming a compact layer; conidia abundantly produced in succession, becoming aggregated in masses, hyaline, guttulate, unseptate, lunate, 17.5–21 × 2.5–3 μ.

The generic name, *Schizotrichella,* was chosen in order to indicate generic relationship to *Schizotrichum.* The two genera differ primarily in the character of the spores. The spores of *Schizotrichella* are unseptate and lunate and the spores of *Schizotrichum* are septate and straight or curved. The spores of *Schizotrichella* are smaller than those of *Schizotrichum,* but this, of course, is not a generic character. The new genus is in the Tuberculariaceae.

Chir. Cultured from *Cantherellus odoratus* collected above Boquete 2 Aug. 1952. GWM *8791,* TYPE, in herb. SUI, Iowa City.

Neottiosporella radicata sp. nov. FIGS. 1–3.

Sporodochiis albis, piriformibus vel subglobosis, superficialibus, subgelatinosis, plerumque radicatis, 250–750 μ diam.; setis hyalinis vel dilute fumosis, septatis, 75–90 × 5 μ; conidiophoris obsoletis; conidiis dense conglutinatis, hyalinis, continuis, lunatis, 13–17 × 2.5–3 μ, spinis hyalinis, 5 × 1 μ, utrinque fugaceis.

Sporodochia white, pyriform to subglobose, superficial, subgelatinous, attached by a dark point, often with a dark, root-like stalk immersed in substratum, 250–750 μ in diameter and attaining the same height; base surrounded by hyaline or smoky, septate hairs, 75–90 × 5 μ, which become detached and are carried up with the spore mass as it enlarges; conidiophores not seen; conidia densely agglutinated in the gelatinous mass, hyaline, continuous, lunate, the body of the spore 13–17 × 2.5–3 μ, each end tipped by a slender spine, 5 × 1 μ, the spines falling off with age.

The genus *Neottiosporella* was proposed by von Höhnel (**4**) in 1923. No description was given, but by following out the key characters it is possible to compile a description. In this way the genus

was keyed out in Clements and Shear (**1**), the name being shortened to *Neottiosporis*. Furthermore, no species was designated. Graniti (**2**) described a species, *N. Triseti* Graniti from Northern Italy, which thus becomes the type and hitherto the only representative of the genus. Graniti also supplied a formal Latin diagnosis of the genus.

The fungus described here as new, *N. radicata*, represents a second species to be referred to the genus. It differs from *N. Triseti* mainly in the larger spores and the smaller and paler setae and the shorter, more slender spines, at the tips of the spores.

This distinctive fungus appeared but once on the same bark that produced, among other things, the new genus, *Umbellula*, of the Dematiaceae. It was readily secured in pure culture, and fruits best on fragments of dried blue grass covered with plain agar. The invisible mycelium often reaches bits of blue grass immersed in the agar and sends up a black, rhizomorphic strand to the surface, where the fructification forms. As the agar dries, this may remain as a black stalk but there is no suggestion that such a stalk would occur in nature. However, the original fruitings, and those developed on the fragments which remain at the surface of the agar, do have a narrowed, stalk-like base.

BC. Cultured from bark collected 22 Aug. 1952; put in moist chamber 19 Sept. 1952. GWM *8726*, TYPE, in herb. SUI, Iowa City.

I wish to extend my appreciation to Dr. Donald P. Rogers for checking and correcting the Latin diagnoses.

This work was done in the Mycological Laboratory of the State University of Iowa under the supervision of Professor G. W. Martin.

SUMMARY

Twenty-eight species of hyphomycetes are listed as isolates from materials collected in the Republic of Panamá and the Panamá Canal Zone. The materials consisted of fragments of palm leaves, angiospermous wood and a termite nest which were placed into moist chambers. Conidial ontogeny was studied in micro-culture slides. As a result of this study one new genus, *Schizotrichella*, and two new species, *S. lunata* and *Neottiosporella radicata*, are described.

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CHAETOSEPTORIA WELLMANII IN MEXICO[1]

WILLIAM D. YERKES, JR.[2]

(WITH 2 FIGURES)

Chaetoseptoria wellmanii Stevenson (*Mycologia* **38**: 530, 1946), a pathogen of the common bean, *Phaseolus vulgaris* L., is here reported from Mexico for the first time.

The leaf spot caused by this fungus was observed throughout the Central Mesa in beans during the summer of 1955. The organism has been reported previously only from El Salvador. Although the Mexican

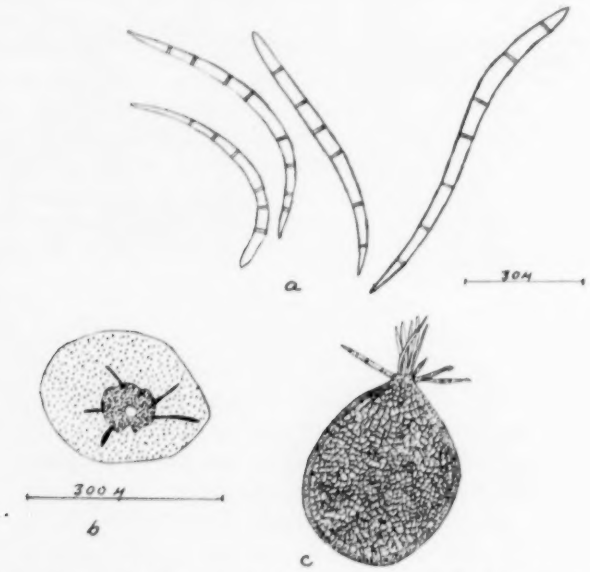

FIG. 1. Pycnidium of *Chaetoseptoria wellmanni*. a. conidia; b. top; c. side view.

[1] Paper No. 46, Agricultural Journal Series of The Rockefeller Foundation. Published as excess pagination at the expense of the Foundation.

[2] Assistant Plant Pathologist with the Mexican Agricultural Program of The Rockefeller Foundation.

material differs somewhat from the type description, I consider it co-specific with the type. The two collections are compared below.

Origin	Diam. of pycnidium	Size of ostiole	Conidia	Number of septae	Size of setae
Type	120–170 μ	15–25 μ	75–160 × 2.5–4 μ	—	90–225 × 5–6 μ
Mexico	150–350 μ	30–50 μ	80–140 × 2.5–4 μ	6–8	60–150 × 3–5 μ

The conidia from the Mexican collections are 6–8-septate, mostly 7, as shown with eosin stain (FIG. 1). Stevenson describes the pycnidia as few and scattered in the leaf spots. In the Mexican material the pycnidia are abundant and distributed throughout the leaf spot, but are especially prevalent in the ashy-grey centers. The leaf spots of the

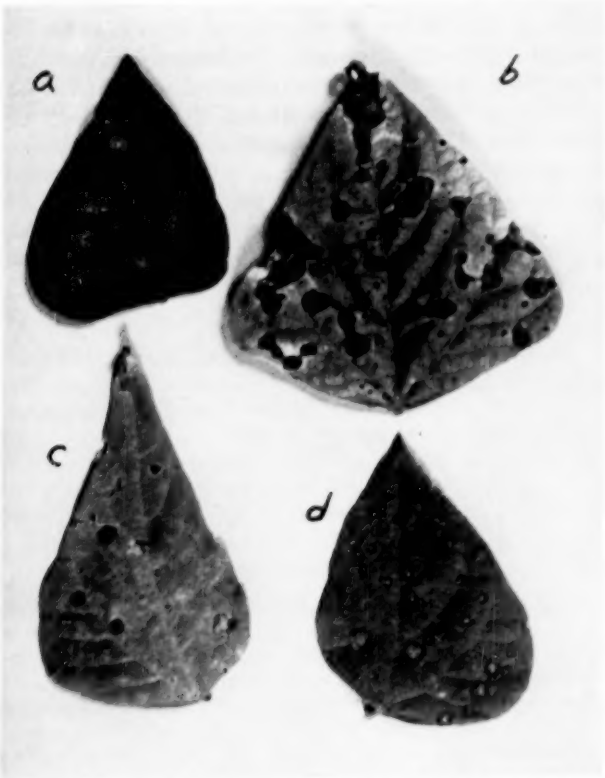

FIG. 2. Leaves of several bean varieties infected with *Chaetoseptoria wellmanii*. a. negro; b. canario; c. bayo; d. amarillo.

Mexican material are faintly zonate. It is suggested that the description of the species be emended as follows:

Leaf spots round to irregular, brown, lighter in the center, subzonate, margin reddish; pycnidia amphigenous, scarce to abundant in the center of the leaf spot, dark-colored, subpyriform; ostiole well-defined, circular, 15–50 μ in diameter; setae straight, 3–9-septate, 3–6 μ diameter, 60–225 μ long, 3–9 in number; conidia hyaline, acicular, straight to curved to flexous, 6–8- (mostly 7-) septate, 70–160 × 2.5–4 μ. In living leaves of *Phaseolus vulgaris* L.

Although some plants were observed to have up to five or six or more lesions per leaf (FIG. 2), the disease caused no apparent reduction in yield.

All types of Mexican beans were susceptible but those of the canario race were most severely attacked. The fungus was encountered on beans throughout the states of Mexico (below 7500 feet), Puebla, Hidalgo, and in portions of Michoacán and Querétaro.

The Mexican collections are deposited in the herbarium of the Office of Special Studies of the Mexican Ministry of Agriculture at Chapingo, Mexico, in the herbarium of the Bureau of Plant Industry of the United States Department of Agriculture at Beltsville, Maryland, and at the State College of Washington, Department of Plant Pathology, Pullman, Washington.

SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE. X [1]

RODERICK SPRAGUE [2]

(WITH 1 FIGURE)

The present article in this series (**8**) is based on recently made noteworthy collections from the Far West plus scattered material either sent for determination or left over from Alaskan collections (**15, 16**). One four-thousand-mile trip into the southwestern United States in June, 1955, encountered predominantly dry conditions. Another shorter trip in the Olympic National Park, Washington, and vicinity proved more profitable. Still later, in August, collections in the Wallowa Mts., Oregon, yielded a considerable number of interesting collections.

Cylindrosporium andropogoni Sprague & Rogerson sp. nov. FIG. 1, A

Maculis fulvis, elongatis v. diffusis, acervulis erumpentibus, hypophyllis, chlorinis, hyphis hyalinis, septatis, aggregatis; conidiophoris hyalinis, compactis, clavatis, 15–25 × 1.5–3.0 μ; sporulis hyalinis v. chlorinis, massa lentis, filiformibus falcatis v. lunatis v. rectis v. vermiformibus, 12–25 × 1.2–3.0 μ, 0- to 1-septatis.

Spots tawny, shading to deeper red-brown or dull copper, elongate and, in the main, delimited by leaf veins although some lesions are diffuse or covering the entire breadth of the leaf; acervuli erumpent, superficial, hypophyllous, yellowish to nearly hyaline, hyphae nearly hyaline, septate, conidiophores compacted, club-shaped, 15–25 × 1.5–3.0 μ, spore masses sticky, drying to obscure scaly residues, hyaline to light yellow-tinted, spores filiform-falcate or lunate, sometimes vermiform or straight, slightly thicker on one end than the other, 12–25 × 1.2–3.0 μ, 0- to 1-septate, many are only 14 × 1.6 μ.

[1] Scientific Paper No. 1480, Washington Agricultural Experiment Stations, Pullman. Project No. 449.

[2] Pathologist, Washington State College Tree Fruit Experiment Station, Wenatchee. The aid of Mary Willis Sprague in collecting and preserving material during the June trip is greatly appreciated. During the July travel I accompanied C. G. Shaw and his class in Field Mycology, and to him and his students I wish to express appreciation for aid in collecting specimens. Permission to collect in the Park was very kindly given by Park officials. Specimens collected there are filed in the Mycological Herbarium, Washington State College, under WSP accession numbers.

KANSAS: Manhattan, August, 1953, on living leaves of *Andropogon gerardi* Vitman (*A. furcatus* Muhl.), in grass breeding nursery, Kansas State College, R. Pickett, TYPE: KSC *R3678;* WSP *37399*.

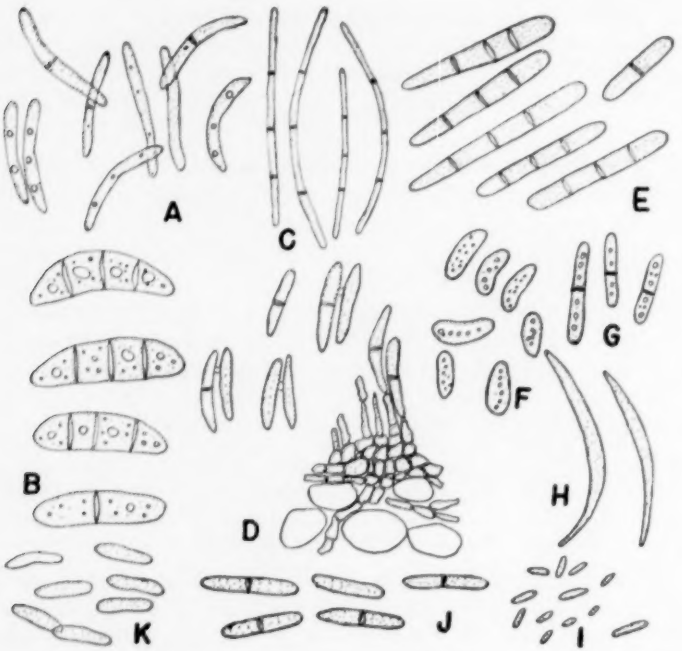

FIG. 1. A. Conidia of *Cylindrosporium andropogoni*, type. B. Pycnidiospores, of *Hendersonia* sp. on *Stipa* sp. 40 km s. of Durango, Mex. (E. Hernandez coll.). C. Pycnidiospores of *Septoria anthoxanthina* on *Anthoxanthum odoratum*, Gold Beach, Oregon (WSP *37456*). D. Conidia of *Gloeocercospora alascensis* on *Elymus glaucus*, Dosewallips River, Wash. (WSP *37466*). E. Pycnidiospores of *Hendersonia culmicola* on *Melica imperfecta*, San Bernardino Mts., Calif. (WSP *37478*). F. Conidia of *Gloeosporium meinersii* on *Phleum alpinum*, Hurricane Ridge, Olympic National Park, Wash. (WSP *42027*). G. Pycnidiospores of *Septoria nodorum* on *Festuca subulata* near Potlatch, Wash. One-septate developing phase (WSP *42041*). H. Pycnidiospores of *Selenophoma donacis* var. *linearis* on *Festuca reflexa*, south of Boise, Idaho (Meiners and Purdy, WSP *42013*). I. Microspores of *Septoria tenella* on *Festuca occidentalis*, Olympic National Park (WSP *42085*). J. Same as G but spores slightly more mature. K. Pycnidiospores from type of *Phyllosticta digitariae*.

All figures drawn with the aid of a camera lucida and reduced to × 1000 in reproduction.

C. T. Rogerson sent this material to Wenatchee requesting determination. He stated that "elongate pustules form beneath the epidermis and eventually break through exposing an elongate yellowish mass (crust-like when dry). Under the microscope this appears to be composed of a tightly compacted layer of clavate conidiophores (15–23 × 1.5–3.0 μ). . . ." The writer decided after considering several genera to ask the advice of Lindsay S. Olive, who has had experience with *Ramulispora* and *Glococercospora*. Olive stated that it was not assignable to either of these genera and suggested that it might be placed in *Cylindrosporium*. After further study and conference with Rogerson, this assignment appears to be logical. The aid of Dr. Olive is greatly appreciated.

Phyllosticta digitariae sp. nov.

Maculis nullis; pycnidiis erumpentibus, nigris, pseudoparenchymaticis, ostiolatis, subglobosis, 60–100 × 58–100 μ; pycnidiosporulis copiosis, cylindraceis v. curvulicylindraceis, hyalinis, 8–9.5 × 2.2–2.6 μ.

Spots none, pycnidia erumpent in dead brown tissue, black to very dark brown, pseudoparenchymatous, appearing almost sub-carbonaceous, ostiole very small, subglobose, 60–100 × 58–100 μ; pycnospores exuded copiously in water mounts, cylindrical or sometimes slightly bent to appear faintly bean-shaped, hyaline, with none or very tiny terminal oil drops, 8–9.5 × 2.2–2.6 μ.

UTAH: Santa Clara, June 25, 1955, in basal sheaths of *Digitaria ischaemun* (Schreb.) Schreb. ex Wimm., near the ground line, R. and Mary W. Sprague, TYPE: WSP *42178*.

This fungus is morphologically closest to *P. bromivora* Sprague but the spores are smaller (compare FIG. 1, K of this article with reference **14**, Fig. 1, C). It was associated with *Helminthosporium sorokinianum* Sacc. The host was growing along the roadside in an irrigated, originally desert region. The host was mixed with *Hordeum stebbensii*. The only other reports of *Phyllosticta* on *Digitaria* is one by Weiss (**22**) of *P.* sp. causing a leafspot on *D. violascens* Lk. from Kentucky and *P. rogleri* Sprague on *D. sanguinalis* (L.) Scop. from Albia, Iowa (**13**, p. 196 and Fig. 19, C). Weiss's report gives no morphology while *P. rogleri* has larger spores which are ellipsoid.

GLOEOCERCOSPORA ALASCENSIS Sprague (**15**) was recently found by P. M. Halisky and the writer on *Elymus glaucus* Buckl. in the Olympic National Park, Washington, growing in a meadow about one-third mile above the falls on the Dosewallips River (WSP *37466*). When we examined the material in the field, the young lesions were white linear

streaks not averaging more than 1.5 mm long. They could be mistaken for the early stages of powdery mildew. The slightly raised masses were sub-cottony but semi-gelatinous when wet. The lesions later increased in size, becoming buff, and measuring as much as 1.0× 0.5 cm. The spores are similar to those from the Alaskan type on *Trisetum*, 13–17 × 1.6–2.1 μ, and most of them were fused basally in pairs (FIG. 1, D). Although on a different host, this fungus appears to be the same as that from Alaska. The habitat was a small meadow and brushy area along the river at an elevation of about 1500 feet.

COLLETOTRICHUM GRAMINICOLA (Ces.) Wils. occurred on a few leaves of *Poa stenantha* Trin. growing in the shade at Sandy Cove, Glacier Bay, Alaska (WSP *37333*). The habitat is unusual, the grass being a relic from earlier succession in the deglaciated area. There is no other collection of anthracnose on this host. No doubt this particular grass is not susceptible to the fungus except under conditions adverse to the growth of the grass. Mrs. Xerpha Gaines verified the determination of this host.

Colletotrichum graminicola also occurs in streaks on leaves of *Glyceria pauciflora* Presl near Mirror Lake, Medicine Bow National Forest, Wyoming, collected August 11, 1948. The material is poor, this host also being apparently resistant to the parasite. There is no report in the western check list (**19**) of *Glyceria* parasitized by *Colletotrichum*. Our record of *Ellisiella caudata* on this host, however, is in error; the fungus is more likely *C. graminicola*.

CERCOSPORELLA POAGENA Sprague occurs in typical buff eyespots on *Poa pratensis* L. at Milepost 7, Hurricane Ridge Road, Olympic National Park, Washington, July 23, 1955 (WSP *42010*). Except for the recent report from Hoonah, Alaska (**15**), this is the only report outside of Oregon, where the fungus was first found.

SPERMOSPORA SUBULATA f. CILIATA Sprague occurs sparingly on elliptical brown lesions of *Festuca elatior* L., seven miles west of Port Angeles, Washington (WSP *37500*). This is a newly reported host for the fungus.

RHIZOCTONIA SOLANI Kuehn causes a well-defined eyespot lesion on the culms of *Dactylis glomerata* along the Elwha River just outside the entrance to the Olympic National Park, Washington. This is the virulent race of the fungus found along the coastal region in Oregon and Washington (**13**, pp. 132–134). The host is new to Washington. The specimen was collected by P. M. Halisky and the writer.

SCOLECOTRICHUM GRAMINIS Fckl. causes the common brown leaf streak on the culm leaves of *Poa epilis* Scribn. along the Hurricane

Ridge Road in the Olympic National Park, Washington (cf. WSP *37492*). Much of the material collected on July 22–23 was immature.

FUSARIUM MONILIFORME var. SUBGLUTINANS Wr. & Rg. was determined by W. L. Gordon as present in a sheath spot on *Hordeum brachyantherum* Nevski collected at Gettysburg, Washington (WSP *42029*), on the beach. Dr. Gordon cultured the fungus from the fragment sent to him. We have no record of this fungus occurring on this grass.

In our original description of GLOEOSPORIUM MEINERSII Sprague (**11**) we failed to illustrate the conidia. In a later discussion of the fungus on *Phleum alpinum* (**14**) we also neglected this point. At this time therefore we are including line drawings of typical spores from another collection on *Phleum alpinum* (WSP *42027*) from Hurricane Ridge Lodge, Olympic National Park, Washington (FIG. 1, F). The spores are borne in large quantities in tawny blotches and streaks. The fungus was also found on *Phleum pratense* at Enumclaw not far from the type locality (WSP *42091*). The obscure species apparently is not uncommon.

FUSARIUM NIVALE (Fr.) Ces. is associated with a necrosis of leaves of *Cinna latifolia* (Trevir.) Griseb. in the Snoqualmie National Forest near Mt. Rainier, Washington (WSP *42137*). The lesions are insect-riddled, and the exact cause of the injury is uncertain. From the paucity of spores, it would appear that the fungus was not primary.

OVULARIA PUSILLA (Ung.) Sacc. & D. Sacc., the subject of a recent revision (**17**), occurs on an additional host, *Phleum alpinum* L., along the bank of a large creek at Tlingit Point, Glacier Bay, Alaska (WSP *37319*). Most of the spore development had apparently occurred in the wet packet collected during pouring rain and delayed in drying. Typical eyespot lesions were found on this new host genus. Spores were small, few and immature.

Ovularia pusilla is common in the Olympic National Park, Washington, on a number of hosts, especially *Elymus glaucus*, *Melica subulata* and *Bromus vulgaris* (Hook.) Shear. The last two mentioned are newly reported hosts for the state of Washington (WSP *42053* and *42084*). *O. pusilla* was also exceedingly common on an awned form of *Agrostis alba* L. in the woods along the west fork of the Wallowa River, Oregon (WSP *42165*).

HELMINTHOSPORIUM (?) CATENARIUM Drechsl. was associated with an obscure mold on basal leaves of *Aira caryophyllea* L. at Port Orford, Oregon, June 19, 1955 (WSP *37454*). The few spores seen were yellow, tapering, somewhat subulate and up to $85 \times 14\,\mu$ in diameter. Somewhat similar but even scantier material was seen on this grass at

Gamm Camp, Dosewallips River, Jefferson Co., Washington (WSP *42096*). Until better material is found, this fungus is assigned to *H. catenarium*. We can find no reports of *Helminthosporium* on *Aira* although we have reports of the genus on *Deschampsia* (**13**, p. 398 and **19**, p. 58). In fact, the *Helminthosporium* on *D. elongata* is not uncommon near Chinook Pass, Washington (WSP *42105*).

Another collection of HELMINTHOSPORIUM was found on *Cynosurus echinatus* L. at Gold Beach, Oregon (WSP *37455*). The spores are tapering, cylindrical, up to 120 × 7 μ. It differed from *H. catenarium* on *Aira* in the longer, narrower, dark brown conidiophores. Those on *Aira* were yellowish, short, relatively stout. The fungus is closer to *H. graminum* than to *H. catenarium* or *H. dictyoides*. The fungus is associated with a faint mold on the muddy basal leaves of the grass. This is the second report of a fungus on this grass from western America, the other being *Phialea*, also from Oregon (**19**, p. 55).

HELMINTHOSPORIUM STENACRUM Drechsl. and ASCOCHYTA SORGHI Sacc. were noted on dead basal leaves of *Agrostis humilis* Vasey at Horseshoe Lake, Wallowa Mts., Oregon. Both fungi are new to this dwarf alpine (WSP *42130*). The *Ascochyta spores* were small, 12–15 × 2.1–2.5 μ and the material was limited to a few pycnidia. Until better material is available for detailed study, this fungus can be called *A. sorghi*. The *Helminthosporium* was widespread in the Upper Basin of the Wallowas.

HELMINTHOSPORIUM TRITICI-REPENTIS Died. appears to be the main cause of a common leafspot of *Elymus glaucus* along the West Fork of the Wallowa River, Oregon. This is the first report of this fungus from Oregon on this host although it occurs widely on it in Washington.

HELMINTHOSPORIUM VAGANS Dreschl. causes a brown spot on leaves of *Poa trivialis* at Silver Springs Forest Camp, Snoqualmie National Forest, Mt. Rainier area, Washington, July 24, 1955 (WSP *42067*). This is the first report of this fungus on this species of bluegrass, at least from the western United States. The conidiophores and spores are fairly typical for *H. vagans* but the lesions lack the purple-brown appearance often seen on *P. pratensis*. Another collection on the same host was found at Gamm Camp, Dosewallips River, Jefferson Co., Washington (WSP *42068*).

Helminthosporium vagans was found on still another new host, *Poa palustris* L., five miles south of Potlatch, Mason Co., Washington (WSP *42032*). The fungus occurred on dead fuscous leaves. The single conidiophores bore yellow, 3–7-septate cylindrical spores 40–110 × 13–14.5 μ.

HENDERSONIA sp. was noted on *Stipa* sp. collected 40 km south of Durango, Mexico, on September 19, 1946, by E. Hernandez. This material showed ostiolate pycnidia somewhat linearly imbedded in black stromata 600–1200 × 500–650 μ. Some of the spots on the sheaths and culms contain mature *Phyllachora* sp. with uniseriately arranged elliptical ascospores with accompanying hyaline linear spermatia. There is no report available to me of *Phyllachora* on *Stipa* from either Mexico or the United States (cf. **4**, **6**, **13**). This collection was sent on to J. J. Stevenson with a request that it be given to C. R. Orton. This occurred only shortly before we heard of the death of Dr. Orton. What disposal was made of the material is not known. One glass slide containing the *Hendersonia* pycnidia and spores is filed in the Mycological Herbarium, State College of Washington, Pullman, as WSP *37307*. The pycnidiospores are abundant, light brown, sub-cylindrical or subcultiform to nearly allantoid, 3-septate, 19–22 × 6.0–6.5 μ when mature (FIG. 1, B). Several years ago we discussed a similar situation involving *Hendersonia* sp., apparently parasitic on *Phyllachora* on *Muhlenbergia* (**14**). Orton (**4**) pointed out the nearly insurmountable difficulty of determining the genetic connection of the forms associated with *Phyllachora*. Greene (**3**) has indicated the same problem with *Davisiella clymina* (Davis) Petrak. The last-mentioned fungus was originally considered to be a conidial form of *Phyllachora*. It was later called a parasite on the *Phyllachora* by Petrak (**5**) and recently Greene relegated it to the status of an associate or competitor of the *Phyllachora*. Perhaps this is the situation in the case of the *Hendersonia* from Mexico. While it may be undescribed, it is more likely close to *Hendersonula aristidae* (Schw.) Ellis. Since the writer is still working on the *Hendersonia* spp. on Gramineae, he prefers to leave this specimen assigned only to the genus.

Members of the genus *Hendersonia* are seldom parasitic, and then only weakly so (cf. **13**, pp. 172–173). A collection of *Hendersonia culmicola* Sacc. on *Agropyron subsecundum* var. *andinum* collected on Logan Pass, Montana, appears on first glance to be actually causing small brown spots on pale green leaves of the host. The fungus is abundantly sporulating on lower leaves and sheaths and causing the spotting and stippling on adjacent leaves. The fungus was probably favored by frosts at this high altitude. It was collected on August 27, 1954.

Hendersonia culmicola and *Septoria nodorum* Berk. occur in some confusing material on *Melica imperfecta* Trin. in the San Bernardino Mts., California. A specimen (WSP *37485*) collected at the 2000 ft

level shows brown blotches containing golden brown pycnidia bearing 1-septate hyaline cylindrical spores 16–20 × 3.0–3.3 μ. These are the *Ascochyta* phase—that is, immature spores of *S. nodorum*. However, in some material collected on this host at the 3,500-ft level in the same mountains, some of the spores are light brown, tapered slightly towards one end, 3-septate and 26–33 × 3.2–4.1 μ (WSP *37478*). They appear to occur in the same type of pycnidia as specimen *37485*. Developing or different 1-septate hyaline spores occur in different but associated pycnidia in collection *37578*. I believe that the brown spores (FIG. 1, E) are fairly typical *H. culmicola*, while the 1-septate hyaline ones in both specimens are *S. nodorum*, the cause of the blotch. Usually developing spores of *H. culmicola* also show yellow tints, while those of the *Ascochyta*-like phase of the *Septoria* are hyaline. However, on *Festuca myuros* L. (WSP *37480*) growing with collection *37478* we have 1-septate yellow spores which are usually called *H. culmicola* var. *minor*. This is widespread on fescue grasses, the material on *F. myuros* representing only an additional recorded host.

Besides the *Septoria* and *Hendersonia* found in the San Bernardino Mts., California, we encountered similar material on *Bromus sterilis* L. (WSP *37483*) but which had mature hyaline, 1-septate spores. This is typical for ASCOCHYTA SORGHI Sacc., a new host for this common leafspot.

Besides above-mentioned reports of *Ascochyta sorghi*, we have some material on *Bromus secalinus* var. *velutinus* (Schrad.) Koch which is assigned to that fungus. The collection was made along the beach at Gettysburg, Washington (WSP *42122*). The spots are chocolate brown to black. The hyaline, 1-septate spores are 11–16 × 3.0–3.9 μ. The color of the lesions suggest *Stagonospora bromi* Sm. and Ramsb., but the spores appear mature and assignable to *A. sorghi*.

Ascochyta phleina Sprague has narrow spores, 11–16 × 1.5–2.2 μ in the type on timothy from Minnesota (**10**, **13**). John Webster sent material from Sheffield, England, on *Dactylis glomerata* L. with spores 11–18 × 1.8–2.5 μ. Still more recently we obtained a collection from five miles south of Potlatch, Mason Co., Washington, which at first glance appeared similar. A few pycnidia were found in tawny streaks on *Festuca subulata* Trin. The cylindrical spores were aseptate to obscurely 1-septate, 13–17 × 1.7–2.3 μ (FIG. 1, G). They were hyaline but of a bright ice-blue appearance with a number of spherical, bright, hyaline spore inclusions often seen in young spores. Morphologically this collection (WSP *42041*) was referable to *A. phleina*. However, we have a second fragment of *F. subulata* from the same location and

on the same plant that has slightly larger cylindrical spores (WSP *42134*). These measured 12.8–15.4 × 2.2–2.9 μ averaging mostly 14 × 2.6 μ (FIG. 1, J). This specimen appears to be slightly more mature than WSP *42041*. Symptoms, location and host are identical, and obviously both collections belong to the same species. I would rule out both *A. phleina* and *A. sorghi* and call these collections very young material of *Septoria nodorum*.

Septoria nodorum was also found on *Agropyron repens* near the mouth of the Dosewallips River, Washington. This grass must be somewhat tolerant of this fungus, because this is the only collection west of North Dakota of blotch on this host. The lesions are light brown. The spores are 0–1-septate, 17–22 × 2.3–2.6 μ. They are immature (WSP *42148*).

Abundant material of *Phyllachora graminis* (Pers.) Fckl. was collected on *Elymus glaucus* Buckl. by P. M. Halisky, September 16, 1955, at Bassett Bay, Stuart Island, B. C. (WSP *42094*) and along the shore of Eagle Lake, Stuart Island (WSP *42093*). We have no record of this fungus on *E. glaucus* from British Columbia.

LEPTOSPHAERIA sp. with 5–7-septate spores occurs in linear lesions on living leaves of *Phalaris arundinacea* L. near Potlatch, Washington (WSP *42064*). The same fungus also occurs in saprophytic material in the same collection but was filed as a separate accession (WSP *42065*). The lesions showing parasitic tendencies are about 1 mm wide and 1–2 cm long. They lie in the main body of healthy leaves or along the margin. The perithecia are few, about 200 μ diameter and contain linear asci 90 × 14 μ and spores averaging 28 × 4.2 μ. The spores are somewhat smaller than those of *L. herpotrichoides* de Not. (cf. **13**, p. 83). They have no resemblance to the 3-septate spores of *L. avenaria* Weber. The fungus, while probably parasitic, is not described as new at this time. It should be compared with the large-spore saprophytic species of *Leptosphaeria* on Gramineae. Also noted on straw-colored leaves of the *Phalaris* were spores of *Stagonospora simplicior* Sacc. and Berl. (WSP *42063*).

MACROPHOMA PHLEI Tehon and Stout was found on dried leaf terminals of *Elymus cinereus* Scribn. and Merr. (*E. condensatus* Amer. Auct.) growing at 7000 ft on Pequop Pass, Nevada, June 27, 1955. The material was fragmentary. The spores were immature, 12–18 × 6.5–7.4 μ, pellicular to ovate with typical opaque white contents. The fungus was in a specimen heavily infected with *Rhynchosporium secalis* (Oud.) J. J. Davis (WSP *37428*). The *Rhynchosporium* was new to Nevada and the *Macrophoma* is on a newly reported host.

SEPTORIA ANTHOXANTHINA Gz. Frag. was collected on leaves of *Anthoxanthum odoratum* L. at Gold Beach, Oregon (WSP *37456*), June 19, 1955. The pycnidia were brown, ostiolate, typically elongate, 85–135 × 65–78 μ. The hyaline filiform spores were faintly 3-septate, straight or curved, 26–29 × 1.2–1.5 μ (FIG. 1, C). The type of this fungus from Spain on *A. amari* had pycnidia 90–120 μ in diam. and filiform, guttulate spores 25–30 × 1.2–1.5 μ (**2**). The Oregon collection appears identical. We can find no mention of this fungus except from Spain. The Gold Beach material occurs with the omnipresent *Colletotrichum graminicola*. We also noted some filiform spores with secondary appendages but their origin was not determinable. All of the spores actually seen emerging from pycnidia were filiform and without branching. *S. anthoxanthina* is similar to *S. poliomela* Sydow (**9**; **13**, pp. 251–252) but the spores are more often obscurely tri-septate rather than typically 2-septate. In passing we should note that no material referable to *Phyllosticta anthoxella* Sprague (**8**) has been seen. This fungus has spores only 5–9 × 1.0–1.6 μ borne in small pycnidia. Perhaps this species of *Phyllosticta* is only the microspore stage of *S. anthoxanthina*, but evidence is still lacking. It has never been seen since the type collection was made in Oregon.

A considerable amount of the material reported in this paper has been of borderline cases between genera. One of the most difficult of these was a collection of STAGONOSPORA FOLIICOLA (Bres.) Bub. on *Glyceria striata* (Lam.) Hitchc. collected by Jack Meiners and the writer August 8, 1948, along Buckhorn Creek, Colorado (WSP *37327*). When examined in 1948 the hyaline spores resembled a species of *Stagonospora* or a species of *Septoria* with very coarse spores rather than *Phaeoseptoria festucae* Sprague. After aging in the herbarium, the spores are still hyaline. The 7–13-septate spores measured 45–60 × 5–6 μ. On comparing with some material of *St. foliicola* collected on *Glyceria* the day after leaving Buckhorn Creek (cf. **11**, pp. 500–501), the writer, after some hesitation, has rejected *P. festucae* from consideration and assigns this material also to *St. foliicola*. Along the same line there is some scanty brown blotch material on *G. striata* from the Olympic National Park, Washington (WSP *42005*), which is also referable to *St. foliicola*. The more mature spores are 7- (rarely 8–9-) septate, sometimes strongly constricted at the septa, stiffly curved and up to 50 × 6 μ in size, hyaline. Still later we determined further material on *G. striata* from the vicinity of Potlatch, Mason Co., Washington, as *St. foliicola* (WSP *42124*).

SEPTORIA GLYCERIICOLA Sprague was abundant in the Buckhorn

Creek material which we have discussed above. It was segregated as much as possible into a separate packet (WSP *37328*). We have reported somewhat similar material before (**10**, pp. 183–184; **12**, p. 769). The 0-septate spores (microspores) are hyaline, bacillar, straight or curved, 10–14 × 1.0–1.4 μ, the 1-septate spores are short, filiform-obclavate to bacillar, mostly bent, 14–20 × 1.0–1.4 μ. The lesions tend to be in streaks or long, vague, straw-colored areas rather than in blotches as in *Septoria nodorum* Berk. The abundant pycnidia are obscure. This is summer material, heat-stunted and atypical. *S. glyceriicola* has been differentiated from *S. avenae* by its narrower spores (**13**). We saw a great deal of *S. avenae* on *Glyceria* spp. in the Olympic Peninsula and in the Cascade Range in 1955 but none that we would call *S. glyceriicola*.

SEPTORIA OUDEMANSII Sacc. occurs on leaves and sheaths of *Hierochloë odorata* (L.) Wahl. collected by C. G. Shaw at a cottage then owned by H. C. Greene at Eagle, Waukesha Co., Wisconsin, May 24, 1941 (WSP *37337*, cf. Shaw *234*). Light golden-colored pycnidia with prominent dark ostiolar rings were abundant in tawny lesions. The pycnidia measured 85–140 μ diam. The spores in this material are somewhat narrow for *Ascochyta sorghi* which occurs on sweet vernal grass. They are slightly tapered at either end, 13–20 × 2.2–3.0 μ and appear fairly typical of *S. oudemanssii* as it occurs on *Poa* spp. (cf. **13**, Fig. 42, A).

Seymour reports *S. oudemansii* on *Hierochloë alpina*, an arctic species (**6**), and since Cash (**1**) does not report this grass as a host for *S. oudemansii* from Alaska, nor does Sprague (**18**), it is assumed that this report is probably from Greenland or the northeastern United States. Seymour also lists *S. graminum* Desm. on *H. odorata*. This material has not been located. It is doubted if it is *S. graminum* (**7**). Information on *S. oudemansii* from Wisconsin has been forwarded to Dr. Greene for possible inclusion in his comprehensive reports of state fungi.

SEPTORIA POLIOMELA Sydow is an obscure species which was found four times in 1955 on the unreported host *Deschampsia elongata* (Hook.) Munro, once near Lake Mill, Olympic National Park, Washington (WSP *42009*), and again a few miles away on Hurricane Ridge in the same park (WSP *42081*), both on July 23, 1955; across the state at Field Springs Forest Camp, Asotin County, Washington, on August 15, 1955 (WSP *42076*), and from the West Fork of the Wallowa River, Oregon (WSP *42191*). The filiform spores of WSP *42009* averaged 30–44 × 1.3–1.5 μ, those from WSP *42081* were as long as 71 μ, while

those from the drier area in eastern Washington were smaller, 20–29 × 1 μ. The collection from Wallowa County, Oregon, had spores 21–35 × 1.0–1.2 μ. The same fungus was found on the annual grass *D. danthonioides* at Port Discovery, Washington (WSP *42132*) but this host was noted some years ago in Klickitat County, Washington (**9**). The fungus was also fairly common on yellowed or tan-colored leaves of *D. caespitosa* in alpine meadows in the Wallowa Mts., Oregon (WSP *42155*). The spores in this material are larger than on the smaller hosts and appear identical with those of a collection on this host found in a marsh near Granger, Oregon, about twenty years ago (**9**).

The microsporous phase of SEPTORIA TENELLA Cooke and Ellis occurs on living leaves of *Festuca occidentalis* Hook. in the Olympic National Park. The grass was growing in dense woods at Milepost One, Hurricane Ridge Road (WSP *42085*). The spores are bacillar-like, 4.8–6.5 × 0.8–1.2 μ (FIG. 1, 1). They are too small for *Phyllosticta* sp. which is sometimes seen on *F. subulata*, another woodland species of fescue, and are assignable to *S. tenella* (cf. **13**, Fig. 49). The small pycnidia occur in linear buff lesions on otherwise healthy leaves. *F. occidentalis* is a newly noted host.

Macrospores of *S. tenella* were also seen on Hurricane Ridge on *Festuca ovina* var. *brachyphylla*. The spores were as much as 80 × 1.2 μ (WSP *42121*).

SEPTORIA BROMI Sacc. was noted on necrotic leaves of *Bromus vulgaris* (Hook.) Shear in deep woods at Gamm Camp, Dosewallips River, Washington. The spores measured 35–57 × 1.6–3.0 μ borne in golden brown pycnidia up to 180 μ in diameter (WSP *42086*). The host is new. The spores are large for *S. bromi* but certainly did not have the stiffly javelin shape of *S. jaculella*. Another collection of *S. bromi* was also found a number of miles away from WSP *42086*, this time on *Bromus carinatus* at Holiday Beach on Hood Canal. The tiny pycnidia, 40–55 μ, were clustered on moldy leaf terminals. The 1-septate filiform spores were 23–33 × 1.2–1.4 μ. While this material would appear to be very distinct from that on *B. vulgaris*, the difference is due to environment. The summer material on *B. carinatus* occurs on a dry beach as contrasted with the rain-forest habitat of the specimen from *B. vulgaris*. Summer material of *S. bromi* is more often like the Holiday Beach collection. This is the only report that we have of *S. bromi* on *B. carinatus*. *S. jaculella*, is, of course, well known on this host. The specimen of *S. bromi* from Holiday Beach represents dubious parasitism. In earlier cross inoculation trials we were never able to infect *B. carinatus* with *S. bromi* (**9**).

PHAEOSEPTORIA FESTUCAE Sprague was found on leaves of *Phleum alpinum* L. at Tlingit Point, Glacier Bay, Alaska (WSP *37321*). The 7-septate spores were 38–49 × 3.5–4.2 μ, borne in thin-walled pycnidia, 175–200 μ in diameter. The material represents fast-growing saprophytic development under humid conditions during and following collecting. We have reported *P. poae* on *Phleum pratense* from comparable conditions along the seashore in the San Juan Islands (**14**, p. 559). *P. poae*, however, has narrower spores, 2.1–2.7 μ, than the Alaskan collection. *P. festucae* is proving to be a widespread, if seldom abundant, saprophyte on many grass hosts.

STAGONOSPORA SIMPLICIOR Sacc. & Berl. occurs on dead leaf tips of *Stipa lettermani* Vasey along the West Fork of the Wallowa River, Oregon (WSP *42131* and *42179*). *St. simplicior* was also found in abundance on *Stipa coronata* Thurb. in the foothills of the San Bernardino Mts. above San Bernardino, California, June 24, 1955. The sunken pycnidia were found scattered widely over straw-colored leaves of mature plants. The pycnidia averaged from 1 to as many as 10 fruiting bodies per square millimeter of leaf surface. While the fruiting bodies must have been formed in late winter, there were no multiseptate spores present. All that were seen were 1-septate and 21–28 × 6.0–8.6 μ in size. They were, in the main, badly plasmolyzed. However, sufficient numbers were intact to permit accurate spore measurements (WSP *42172*). In 1950 we collected similar material on this host in the hills near San Diego, but the box containing this material was lost in transit. The fungus is probably widespread in the chaparral on this host. The areas where we have found it have been very dry at the time of collection in early summer. See also earlier report on *Stipa* spp. (**12**).

STAGONOSPORA VEXATULA Sacc. occurs on straw-colored lesions on living plants of *Poa marcida* Hitchc., growing in dense woods at the foot of Hurricane Ridge Trail, Olympic National Park, Washington (WSP *42110*). The light brown pycnidia are sub-superficial and bear coarse, cylindrical, yellow-hyaline spores, 41–45 × 5.5–6.3 μ. The spores are 5–8-septate, mostly 7-septate, and are strongly constricted at most of the septa. The fungus resembles material of *St. vexatula* on *Deschampsia caespitosa* from Idaho (**11**, **13**, **14**). The host from the Olympics, *Poa marcida*, is a delicate woodland species which is unreported in grass disease check lists (cf. **13**, **18**, **19**).

SELENOPHOMA BROMIGENA Sacc. on *Bromus carinatus* Hook. and Arn. occurs on Hurricane Ridge, Olympic National Park (WSP *42051*), and near Sequim, Washington (WSP *42113*). This coastal

material occurs on old leaves and on the heads. The spores are small, about 14 μ long and are strongly curved.

SELENOPHOMA DONACIS var. STOMATICOLA (Bauml.) Sprague & A. G. Johnson was seen on leaves of *Elymus glaucus* Buckl. on the beach at Gettysburg, Washington (WSP *37493*). The spores were 9–13 × 1.6–2.0 μ and some were blunted, somewhat as in *S. obtusa*. The material is atypical for either *S. obtusa* or *S. donacis*. It reminds one of coastal material of *S. donacis* var. *stomaticola* recently reported on *Puccinellia* from Alaska (**16**, pp. 253–254, Fig. 1, A). The Gettysburg material is associated with leaf rust and *Rhynchosporium* which may also account for its atypical aspect. We do, however, have some good material of *S. donacis* var. *stomaticola* on *Phleum alpinum* L. from Hurricane Ridge, Olympic National Park, Washington, in the same general area but in a very different habitat (WSP *42136*). One would expect this fungus to be widespread on this alpine host but, except for its prevalence on Logan Pass, Montana, and this specimen, we have found this alpine grass free of it to date.

SELENOPHOMA DONACIS var. LINEARIS Sprague & A. G. Johnson was found on a new grass host, *Festuca reflexa* Buckl., some 25 miles south of Boise, Idaho, July, 1955, by J. P. Meiners and L. H. Purdy. The var. *linearis* was created (**20**) to dispose of some fragmentary material on *Agropyron spicatum* collected near Lyle, Washington, about 19 years ago. It was differentiated by its comparatively long, narrow, curved spores, 20–34 × 1.8–2.5 μ. The material from Idaho which Meiners sent to me has comparable spores, 26–32 × 2.1–2.7 μ (FIG. 1, H). Its tendency towards boomerang shape places it closer to *S. donacis* proper than var. *stomaticola*. While probably racially distinct from the type of var. *linearis*, it is for all practical purposes assignable to that obscure variety. The black pycnidia occur on straw-color bleached leaves of this dwarf annual grass. Later, Meiners, while sorting out smut material from the Boise collection, found a number of other plants of *F. reflexa* affected with the *Selenophoma*. These (WSP *42161*) specimens had the same kind of spores except that they ranged up to 3.3 μ broad and some were 1-septate; in fact one spore was noted with three septations. Certainly this variety, as indicated by all of Meiners's and Purdy's material, is only a variant of the *S. donacis* complex. It is difficult to understand why these spores in this desiccated desert material on this puny annual fescue should have some septate spores unless a summer rain had fallen causing partial germination. Meiners also picked out several plants of *F. pacifica* Piper from

the Idaho collections which showed the *Selenophoma*. This (filed as WSP *42162*) had spores up to 30 × 3.3 μ wide and a few were 1-septate. The pycnidia were mostly empty and the relatively few spores seen were sometimes abnormal. One seen was 1-septate and 15 × 4.5 μ in size. Like those on *P. reflexa*, the pycnidia were on bunt-infected plants.

Erysiphe graminis DC., a very common fungus, was found on *Poa trivialis* L., an unreported host from the United States, under trees along the West Fork of the Wallowa River, Oregon (WSP *42164*). *E. graminis* was abundant on *Agrostis exarata* Trin. along Weeping Rock Trail in Zion Canyon, Utah (WSP *42173*). It was noted on *Elymus glaucus* for the first time in Oregon along the West Fork of the Wallowa River (WSP *42190*).

ASCOCHYTA BRACHYPODII (Sacc.) Sprague & A. G. Johnson occurs on leaf tips of *Stipa columbiana* Macoun along a creek one mile below Horseshoe Lake, Wallowa Mts., Oregon (WSP *42174*). The spores in this collection are the same as those illustrated for some material on *Stipa comata* from Hebgen Lake, Montana (**21**, Fig. 1, R). The spores of the Montana collection measured 18–23 × 4.0–4.3 μ, while those from the Wallowa Mts. averaged smaller, 15–20 × 3.0–4.2 μ. The fungus appears to be closer to *A. brachypodii* than to *A. sorghi*, especially in the general shape of the spores and its probable saprophytic nature. The same fungus was also found on *Stipa lettermani* Vasey along the West Fork of the Wallowa River, Oregon (WSP *42179*).

SEPTOGLOEUM OXYSPORUM Sacc., Bomm. & Rouss. causes a leafspot on *Agrostis thurberiana* Hitchc. in a meadow above Douglas Lake, Wallowa Mts., Oregon (WSP *42175*). This material is comparable to that found by Bonar in the high country in Wyoming (**14**). The spores in the Wallowa material are only 21–25 × 4–5 μ but are young, most of them only starting to form septations.

HELMINTHOSPORIUM STENACRUM Drechsl. was found on dead brown areas on leaves of *Agrostis thurberiana* in the same material above discussed. The material was apparently young. The cylindrical spores were nearly hyaline, measuring 60–71 × 13–15 μ. The sporophores were not typical, also apparently young. They were very short and relatively stout, mostly 35–70 × 12–13 μ, yellow-brown. The fungus appeared to be parasitic. It was more common in the collection than the *Septoglocum*. The original slide mount and part of the specimen were separated from WSP *42175* as WSP *42176*. Both specimens should be used, however, in studying either of the fungi since the material was thoroughly mixed, both fungi occurring on the same leaf in some of it. The host is newly reported for this fungus.

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GASTEROMYCETES FROM PANAMA AND COSTA RICA

J. H. B. Garner

(with 4 figures)

Collections of Gasteromycetes were made in Panamá in 1935, 1937, and 1945 by G. W. Martin and in Panamá and Costa Rica in 1952 by G. W. Martin and A. L. Welden. These collections include species previously reported from Barro Colorado Island by Standley (**1927**) and Weston (**1933**) and additional species not previously reported from Panamá. To my knowledge there have not been any previous reports on Gasteromycetes from Cotsa Rica. No attempt has been made to include complete synonymy, but references are given to other names used in the literature cited.

Collection numbers up to *7000* refer to collections by Martin; *7001* and higher by Martin and Welden.

LYCOPERDACEAE

Calvatia candida (Rostk.) Hollós, Termesz. Fuz. **25**: 112. 1902.

This species has heretofore been collected only from the temperate regions and not from the western hemisphere.

Two collections (*6137, 6181*) were made in the Ft. Sherman area, Canal Zone, Panamá.

Calvatia cyathiformis (Bosc) Morgan, Jour. Cin. Soc. Nat. Hist. **12**: 168. 1890.

This species is world-wide in its distribution and has previously been reported from Colombia, Venezuela and the West Indies in the western hemisphere. Dennis (**1953**) cites this species as *C. lilacina* (Mont. & Berk.), P. Henn.

Collections: Balboa, Canal Zone, Panamá (*4167, 4217*).

Lycoperdon subincarnatum Peck, Ann. Rep. N. Y. State Mus. **24**: 82. 1872.

The outstanding characteristics of this species are its pitted inner peridium and scanty, compact, sterile base. It was found growing quite abundantly on decaying wood.

Collections were made in the following localities: Canal Zone: Barro Colorado Island (*3157, 7329, 7692, 7788, 7790, 7873, 7923, 8490, 8521, 8624*), East of Arraiján (*8377a*), Rio Sardinella (*7542*) and Costa Rica: above Palmar Norte (*8277*).

LYCOPERDON SPADICEUM Pers., Jour. de Bot. 2: 18. 1809.

Two collections (*8492, 8577*) were made on Barro Colorado Island, Canal Zone. This is the first time, to my knowledge, that the species has been collected outside of the United States in the western hemisphere.

LYCOPERDON ERICETORUM Pers., Jour. de Bot. 2: 17. 1809.

L. ericetorum Pers. has not been previously reported from Central America. Spegazzini reported it from South America in his Fungi Argentini as *L. furfuraceum* Schaeff. It has been reported from Puerto Rico and Colombia as *L. pusillum* (Batsch.) Fries and from Venezuela as *L. cepaeforme* Bull. These are all synonyms of *L. ericetorum* Pers.

Three collections of this species were made on Barro Colorado Island, Canal Zone (*7368, 7460, 8659*).

LYCOPERDON MOLLE Pers., Syn. Fung. 150. 1801.

The strongly warted spores and dark purple gleba of this species distinguish it from *L. umbrinum* Pers. in which the spores are smooth or finely verrucose and the gleba is olive or umber brown.

Collections were made in the province of Chiriquí, Panamá: near Casita Alta (*8048*), on the trail between Finca Lerida and Casita Alta (*8216*), and in the valley of the Rio Chiriquí Viejo (*2583*). These localities are at an altitude of 1600 to 2200 m.

No collections have previously been made in the western hemisphere south of the United States.

LYCOPERDON PERLATUM Pers., Syn. Fung. 145. 1801.

The single collection (*8199*) was made in the province of Chiriquí, Panamá, on the trail between Finca Lerida and Casita Alta at an altitude of 2000–2200 m.

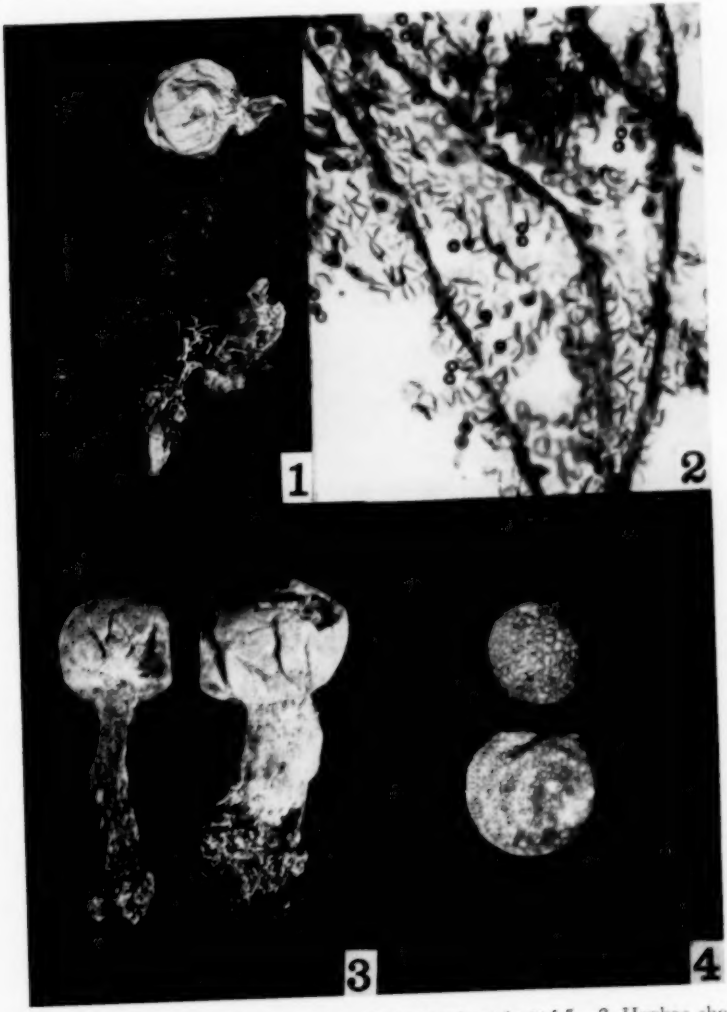
Though quite common in the United States, this species seems to be less abundant in South and Central America.

LYCOPERDON CURTISII Berk., Grevillea 2: 50. 1873.

This species has been reported from Puerto Rico as *L. Wrightii* Berk. & Curt. Aside from this Puerto Rican collection it has not been

previously reported in the western hemisphere outside the United States.

Collections: Panamá: Prov. Coclé: El Valle de Anton, altitude 600–700 m (*2927*) and Prov. Chiriquí: Llanos del Volcan, altitude 1250–1300 m (*2051*).



FIGS. 1, 2. *Morganella mexicana*. 1. Approximately × 1.5. 2. Hyphae showing the filmy membrane in which they are embedded. FIG. 3. *Scleroderma verrucosum*. Approximately × ⅔. FIG. 4. *Caloderma Petrianum*. Approximately × 1.

MORGANELLA MEXICANA Zeller, Mycologia **40**: 651. 1948. FIGS. 1, 2.

The first collection of the species was made in Mexico by C. L. Smith[1] and tentatively assigned to *Lycogala*. After a study of the type specimen, Zeller decided it represented a new genus and gave it the name *Morganella mexicana*.

The characteristics of the genus which differentiate it from *Lycoperdon* are the filmy membranes of which the gleba is composed and in which the capillitium is embedded and the disappearance of the whole gleba at maturity leaving a more or less cupulate empty peridium.

Collections were made in Panamá: Canal Zone, Ft. Sherman area (*6183, 6201*), Republic of Panamá: Prov. Panamá; Rio Tucuman valley 10 k east of Juan Diaz (*3180*); Prov. Coclé: El Valle de Anton, altitude 600–700 m (*2934, 2974*).

In addition to Mexico and Panamá this species has been collected in Colombia (*3440*), as well as in New Jersey.

LYCOGALOPSIS SOLMSII Ed. Fischer, Ber. deut. Bot. Gesell. **4**: 192. 1886.

Previous to the 1935 collection, the only species of *Lycogalopsis* described from the New World were *L. Dussi* Pat. and *L. subiculosa* Lloyd. Martin (**1939**) showed that great variation existed in the degree to which the fructification was immersed in the stroma; therefore, *L. Dussi* Pat., which was described as having fructifications nearly free of the stroma, could not be maintained as a species distinct from *L. Solmsii*. *L. subiculosa* is also a synonym of *L. Solmsii*.

Collections were made at the following localities in the Canal Zone: Barro Colorado Island, where it is extremely abundant (*7053, 7083, 7136, 7356, 7398, 7459, 8694*), Balboa at base of Ancon Hill (*2896*), and Ft. Sherman area (*6026, 6095, 6104*).

This species has also been collected in Surinam, Brazil, Trinidad, Martinique and Bermuda in the American tropics.

GEASTRACEAE

GEASTRUM VELUTINUM Morgan, Jour. Cin. Soc. Nat. Hist. **18**: 38. 1895.

Three collections (*7156, 7643, 7651*) were made on Barro Colorado Island in the Canal Zone, Panamá. Reported also from Brazil and the West Indies.

[1] Not by T. H. Macbride, as stated by Zeller. The collector's name was given incorrectly on the packet sent to Zeller for study.

GEASTRUM SCHWEINITZII (B. & C.) Zeller, Mycologia **40**: 649. 1948.

G. Schweinitzii has previously been called *G. mirabile* Mont. Zeller made the new combination after a study of the type of the genus and species *Coilomyces Schweinitzii* Berk & Curt. showed it to be the same as *G. mirabile* Mont. The specific epithet of Berkeley and Curtis antidates Montagne's and therefore necessitated the new combination.

Collections were made from Barro Colorado Island (*7184*) and Balboa (*2891*), Canal Zone, Panamá. Known also from the West Indies and South America.

GEASTRUM SACCATUM Fries, Syst. Myc. **3**: 16. 1829.

Four collections (*7096, 7172, 7705, 7884*) were made on Barro Colorado Island, Canal Zone, Panamá. Known also from the West Indies and Venezuela.

GEASTRUM TRIPLEX Jungh., Tidskr. Natur. Geshied. **7**: 285. 1840.

Two collections were made on Barro Colorado Island, Canal Zone, Panamá (*7471, 8686*).

GEASTRUM RUFESCENS Pers., Syn. Fung. 134. 1801.

One collection (*8381*) was made in the Canal Zone, east of Arraiján.

Palmer (**1955**) cites van Waveren, Med. Ned. mycol. Ver. **15**: 85–129, 1926, as authority for the statement that this is the correct name for the species commonly referred to as *G. fimbriatum* Pers. As van Waveren's paper has not been seen, the species is here cited under Persoon's name without the appendix "em. van Wav." used by Palmer.

The only other report of this species from the tropics is that of Coker (**1934**) as *G. fimbriatum* from Venezuela.

SCLERODERMATACEAE

SCLERODERMA VERRUCOSUM Pers., Syn. Fung. 154. 1801. FIG. 3.

A close resemblance exists between this species and *S. Lycoperdoides* Schw. Coker and Couch (**1928**) and Ed. Fischer (**1933**) consider *S. Lycoperdoides* to be the North American form of *S. verrucosum*.

S. Lycoperdoides is differentiated by its larger spores, method of dehiscence and the persistence of the hyphae of the tramal plates.

The spores of *S. verrucosum* in the collections from Panamá differ

little in size from those of *S. Lycoperdoides;* however, the peridium of *S. verrucosum* is much rougher than that of *S. Lycoperdoides.*

Collections were made on Barro Colorado Island, Canal Zone (*8594*), and in the province of Chiriquí, Panamá: Casita Alta, altitude 2000–2200 m (*8113, 8167*), trail from Casita Alta to Finca Lerida, altitude 1600–2000 m (*8206, 8208*), and the upper valley of the Rio Chiriquí Viejo, altitude 1600–1800 m (*2080, 2081, 2089, 2183, 2206, 2207, 2208, 2209, 2210, 2222, 2327, 2412, 2492, 2503, 2528, 2549, 2665, 2674, 2709*).

SCLERODERMA CEPA Pers., Syn. Fung. 706. 1801.

The specimens in this collection are immature; therefore no spores are present and the gleba is still white in color. In *S. cepa,* the gleba is usually white until the fungus is about half grown. Other distinguishing factors which seem to indicate that the specimens are *S. cepa* Pers. are the smooth, finely areolated, dark purplish brown peridium and the scanty or absent stem-like base.

The single collection (*8207*) was made near Casita Alta, province of Chiriquí, Panamá, altitude 2000–2200 m.

Previous to this collection, the fungus has been known only from South Africa and the United States.

SCLERODERMA CHRYSASTRUM Martin, Mycologia **46**: 527. 1954.

This is a species described from specimens collected in Panamá by G. W. Martin and A. L. Welden in 1952.

Collections were made on Barro Colorado Island, Canal Zone, Panamá (*7367* (type), *7472, 7696* (paratypes), *7885*).

CALODERMA PETRIANUM Ed. Fischer, Natur. Pflanzenfam. **7a**: 38. 1933. FIG. 4.

The strongly warted peridium, which is composed of broad-lumened hyphae, and the many-loculed gleba serve to set this genus off from *Scleroderma.*

Previous to the collections from Panamá, the genus has been known only from the Eastern Hemisphere.

Collections were made from Panamá: Prov. Colon, East of Colon (*6009*), and from Cerro San Bastarda (*7508*) and Ft. Sherman (*8708*) in the Canal Zone.

Distribution: Malacca, Borneo, Panamá.

NIDULARIACEAE

CYATHUS POEPPIGII Tul., Ann. Sci. Nat. III. **1**: 70. 1844.

Collections were made from Barro Colorado Island (*7158, 7262, 7296, 7430, 7441, 7484, 7580, 7622, 7856*), and Summit (*2871, 2875*) in the Canal Zone, Panamá: valley of the upper Rio Chiriquí Viejo in the province of Chiriqui at an altitude of 1600–1800 m (*2114, 2500*) and Valle Chiquita, 7 k south of El Valle de Anton in the province of Coclé at an altitude of 500–600 m (*3001*).

This species has previously been reported from the West Indies.

CYATHUS STERCOREUS (Schw.) de Toni, Saccardo, Syll. Fung. **7**: 40. 1888.

Collections: Summit, Canal Zone, Panamá (*8257*) and in the Forest Reserve opposite Esquina Experiment Station 35 k east of Palmar, Costa Rica (*8257*).

This species has been reported from Puerto Rico and Venezuela in tropical America.

The identification and discussion of *L. spadiceum, L. ericetorum, L. molle* and *L. Curtisii* have incorporated the nomenclatorial changes adopted by Garner (**1955**).

This work was done in the Mycological Laboratory of the State University of Iowa under the supervision of Professor G. W. Martin.

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OBSERVATIONS ON CHYTRIDIACEOUS PARASITES OF PHANEROGAMS. V. THE OCCURRENCE OF PHYSODERMA BUTOMI AND P. VAGANS IN THE UNITED STATES [1, 2]

F. K. Sparrow

1. Physoderma butomi Schroeter

The common European "flowering rush," *Butomus umbellatus* has been steadily extending its range westward in the northeastern United States for some years. In the Great Lakes region it has become established in considerable numbers in the marshes at the western end of Lake Erie and in the Grosse Isle-mainland area of Monroe Co., Michigan. Indeed, it has been found by this writer established as far north in Michigan as Cheboygan Co., close by Mackinaw Straits. In the southeastern part of the state, in addition to the areas already mentioned, it has also been discovered in Washtenaw, and, particularly, in Oakland Counties.

Schroeter (**1883**) long ago described a species of *Physoderma* on *Butomus* and it has been recorded subsequently by various investigators from several European localities and from North Africa. Furthermore, due to the work of Büsgen (**1887**), considerable information on both the endobiotic polycentric and epibiotic, monocentric, phases was early obtained. The species has not, seemingly, been reported until now from North America. As the host continues its invasion of our swampy areas, we may expect the fungus to become more common and better known here.

The present material was found during a search for various species of *Physoderma* carried on during the summer of 1955. It occurred in considerable quantity at a few sites in southeastern Michigan, notably in Oakland Co. It formed, on the flowering stalks and perianths of the host, dark, scattered pustules. Otherwise, the *Butomus* seemed little affected. The purpose of this note is merely to record at this time the presence of the fungus in our country. Further details of its morphology and biology will be published later.

[1] Acknowledgment is made to the National Science Foundation for its support of this study.

[2] Contribution No. 1057, Botany Department, University of Michigan.

2. PHYSODERMA VAGANS SCHROETER

The second of Schroeter's species to be reported here, *P. vagans*, has been reported as occurring on a wide variety of phanerogamic hosts, representative of the Ranunculaceae, Cruciferae and Rosaceae. That the species is actually so omnivorous as reported seems very unlikely indeed. Future cross inoculation work will undoubtedly indicate its actual host range and the species may well be resolved into several entities, as has been suggested by Fischer (**1892**), Minden (**1915**) and more recently by Karling (**1950**).

Physoderma vagans has been reported from several European stations and has been tentatively described from *Sium suave* (*cicutaefolium*) from the United States (Davis, **1919**). The present material was first found and tentatively identified by Prof. L. E. Wehmeyer and the writer on a collection of *Sium suave* from the vicinity of Ann Arbor made in 1940. The fungus was on both the aerial leaves and the stalks. On the former it produced target-like dark spots each containing a central low pustule, whereas on the hollow stalks, enormous blackish and somewhat iridescent or "chitinous" blisters were formed. Both types of diseased areas were filled with the typical flattened resting spores. The fungus has also been found on material of the host collected in the vicinity of the University of Michigan Biological Station, Douglas Lake, Cheboygan Co., Michigan. It undoubtedly has a wider distribution in our country than the present records from Wisconsin and Michigan indicate.

Further details concerning the species will be given in forthcoming papers.

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NOTES AND BRIEF ARTICLES

A reply and an appeal to Professor Guba

It must seem strange to readers of Prof. Guba's[1] note who are informed of my own papers on the same subject that he has proferred the criticism that I place *undue emphasis on the host plant as a specific distinguishing character* . . . and that *a system of classification independent of hosts is to be preferred* when they well know that I have never, in the slightest degree, made any usage of the host plant as a specific distinguishing character.

It appears, as he explains to me in a private correspondence, that in writing this he meant something else, *i.e.*, the reliance I have put, in creating new species, on differences in biometrical studies, these being, he says, a result of host or matrix relationships.

I may have been misled in specific distinctions. I have confessed that (Bothalia **6**: 379, 1954). But who can claim an untarnished record on that count. Prof. Guba will certainly agree that nobody can, even to being exempt from making major mistakes.

In conclusion, I may say that it is regrettable that in wording his criticisms he used a cryptic language which, if understood in the meaning it seems to carry, is a preposterous misstatement.

However, the main issue is in the treatment of the genera.

I will recall that I have not eliminated the genus *Monochaetia*. I have included some of its species in the *Pestalotiopsis* because they differ from other species of this genus only in having one setulum. My contention is that *Monochaetia*, understood in the meaning that Saccardo has given it, is an artificial genus; incompatible with modern views on systematics of the fungi. A recent examination of Spegazzini's types entirely confirms me in this opinion. Going into details would lead us here too far. Spegazzini cannot be blamed for having included his species in *Monochaetia*. Unless making a revision he could not do otherwise. But, considering the amount of work that has accumulated since Spegazzini's times, what was normal then is no more so now.

It is thoroughly illogical to give generic status to *Monochaetia* (*sensu* Saccardo), which differs from *Pestalotia sensu* Saccardo by one char-

[1] Guba, E. F., *Monochaetia* and *Pestalotia*. Mycologia **47**: 920–921, 1955.

acter and *only one,* whereas *Pestalotia sensu* de Notaris, *Pestalotiopsis* and *Truncatella,* which differ from each other by *a set* of characters, are not given such status. I have explained myself at length, in this respect, in my reply to Servazzi (Bull. Jard. Bot. Etat, Brux. **25**: 191–199, 1955).

Prof. Guba ends up by saying that his aim is a simple, practical arrangement. What is at issue? In taxonomical research, are we catering for the needs of applied botanists or are we endeavouring to find a natural arrangement of plants in related groups at all taxonomical levels?

Prof. Guba's interest in this group of fungi is of a much longer standing than mine and I very readily pay respectful tribute to his many years of patient work. There are rare examples of such devotion and application to the solution of a problem. What I have endeavoured is, in the main, but a contribution to the generic arrangement of this intricate group.

If, after having pondered over my suggestions and weighed my arguments he, in conscience and to the best of his abilities, thinks that they are not to be taken into consideration and that the traditional arrangement is unimpeachable from the taxonomic point of view, then my suggestions are done for.

But, if his main aim is but to cater for the needs of applied botanists, which in my eyes are but fallacious, then he is failing in his life's work. He is missing a unique chance of putting the systematics of this complex group on a sound, lucid and permanent basis. I ask him to consider my appeal in full equanimity before casting the die.—R. L. STEYAERT, Attaché à l'INEAC, Commission pour l'Étude de la Flore du Congo Belge, Brussels, Belgium.

BOLETUS LURIDUS IN NORTH AMERICA

In a recent article [1] W. H. Snell and Esther A. Dick come to a tentative conclusion that "we have in this country either *B. luridus* or a species very close to it."

This statement, according to my recent experience, is correct. Moreover, it can be stated that the true *Boletus luridus* Schaeff. ex Fr., exactly as known to me in Europe since my childhood, occurs in the vicinity of Douglas Lake within the area of the Biological Station of the University of Michigan and east to Cheboygan, all in Cheboygan Co. This species is included in a recent (collections of 1953) list of Boletaceae of that general region (unpublished), and I would have

[1] Notes on Boletes IX. Mycologia **48**: 302–310. 1956.

waited for its publication in connection with other observations from Northern Michigan, had it not been for the hesitation about a positive determination as *Boletus luridus* mentioned in the article cited above, a hesitation for which my former statement ("probably not occurring in America") may be partially to blame. Nevertheless, the statement quoted does not contain an error as written in 1947 since all the authoritative determinations checked upon (Curtis, Coker, Murrill, and many others) had then turned out to be incorrect, and extensive collecting from Maine to Florida had not yielded a single true *B. luridus*. Even now, it is obvious that *B. luridus* is one of the rarest *Boleti* of North America.

Boletus impolitus Fr. may be a similar case. There can be no doubt but that misdeterminations have occurred on a large scale as far as *B. luridus*, *B. impolitus*, even *B. luteus*, and many others have been concerned. I feel that it is rather commendable to be cautious in the assertion regarding existence or non-existence of a species on a continent, especially where new bases for the classification of the species have just been established, and mere mention of the finding of a species cannot always satisfy the critical mind of the monographer. On the other hand, the now established occurrence of *Boletus luridus*, corroborated by exact observations on the basis of modern taxonomic methods, is likewise a remarkable progress in myco-floristic studies, incomparably more valuable now than it would have been before the status of this species had been cleared up in North America.—ROLF SINGER, Instituto Miguel Lillo, Tucumán, Argentina.

LABORATORY REFRESHER TRAINING COURSES

The Communicable Disease Center of the Public Health Service continues its series of refresher courses. Of particular interest to mycologists are five courses dealing with aspects of medical mycology. Laboratory methods in medical mycology, Part 1. Cutaneous Pathogenic Fungi is offered Jan. 7–18; Part 2. Subcutaneous and Systemic Fungi, Jan. 21–Feb. 1 (part 1, or equivalent education or experience a prerequisite); Laboratory Methods in the Study of Pulmonary Mycoses, Feb. 4–15; Laboratory Diagnostic Methods in Veterinary Mycology, Feb. 25–Mar. 1; Serologic Methods in the Diagnosis of Parasitic and Mycotic Infections, Mar. 11–22.

Information and application forms should be requested from Labora-

tory Training Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.

CIVIL SERVICE ANNOUNCEMENT

The United States Civil Service Commission has announced an Agricultural Research Scientist examination for positions in a wide variety of agricultural fields. The salaries range from $4525 to $10,320 a year. The positions are located principally in the Department of Agriculture and the Department of the Interior in Washington, D. C., and throughout the United States.

To qualify, applicants must have had appropriate education plus professional experience. Graduate study may be substituted for experience. Interested persons may obtain further information regarding the requirements and instructions on applying at many post offices, or from the U. S. Civil Service Commission, Washington 25, D. C. They should ask for Announcement No. 58B. Applications will be accepted until further notice.

REVIEWS

GUIDE PRATIQUE DE MYCOLOGIE MÉDICALE, by Jean Coudert. xiv + 364 pp., 8 pl. Masson et Cie., 120, Boulevard Saint-Germain, Paris, VIe. 1955. Price, bound, 6,000 fcs. (about $17.50).

This reference book attempts to simplify medical mycology for the physician and bacteriologist. An admirable degree of clarity is achieved by the format and by uniform descriptions of species of fungi proved or alleged by various investigators to be pathogenic for man. The net result is a compilation which is well presented and will be found useful. However, as a compilation, it lacks a critical evaluation of the validity of species and of actual etiologic relationships. More careful attention to these important items has improved the quality of much of the recent mycological literature.

The introduction is followed by a concise and useful summary table of mycoses. It is difficult to prepare such a table dealing with a complex subject in which there is disagreement on many specific points. Some of the therapeutic measures suggested in the last column of the table, particularly those directed against cryptococcosis (blastomycose généralisée), coccidioidomycosis and histoplasmosis, are generally considered less effective than the first clinical trials indicated.

Part I is a 44-page section in which mycologic methods of study, handling of specimens, composition and preparation of media, preparation of cultures and staining schedules are clearly outlined. Special methods of study suitable for particular groups of pathogens and serologic methods of diagnosis are also discussed. All of the illustrations used in the volume (8 plates of diagrammatic sketches) are placed at the beginning of this section.

Part II lists the fungi of medical importance according to their systematic position and provides a series of dichotomous keys. For purposes of simplification the author has prepared short keys to genera involved in each of several clinical types of disease, keys to genera within families and keys to species within given genera or subgenera. The usefulness of these separate keys could have been increased by page references which would lead the student from the definitive line of one key to the next key to be consulted or to the detailed species descriptions placed later in the section. As it is, one has to leaf through the

section to find the appropriate key and the Index refers only to species descriptions and does not aid one in finding a particular key.

Part III is a systematic description of specific fungi presented with as much uniformity as the data included in the cited publications permit. Here one enters the uncertain area of nomenclature. The author recognizes 75 species and several additional varieties of dermatophytes distributed among 5 genera according to the taxonomic system preferred by the French school. A more generally accepted system of classification admits only 3 genera and some 15 species of dermatophytes, recognizing the majority of binomials in the older literature as synonyms. Inclusion in this compilation of species of doubtful etiologic relationships was mentioned above. For example, 31 species of *Aspergillus*, 23 of *Scopulariopsis*, 16 of *Geotrichum* and 8 of *Penicillium* are accepted as agents of disease. The clinical conditions attributed to these fungi vary from eczematoid lesions of the skin and otitis to pulmonary and bronchial disease. Many of these instances of alleged pathogenicity rest upon single case reports and need confirmation, but the provisional nature of this relationship is not clearly indicated.

There is a three-page glossary of technical terms.—C. W. EMMONS.

BIOLOGY OF ROOT-INFECTING FUNGI, by S. D. Garrett. xi + 288 pp., 1 text figure, 9 tables. New York: The Cambridge University Press. 1956. Price, $5.50.

In this book Garrett presents ideas he had developed during the last twenty-five years of study of the fungi that cause root disease. These fungi are among the large number of species which grow in the soil. Although many of these soil fungi do not actually cause disease themselves, they are of importance in this type of study since they form a part of the association in which root disease fungi must live and are organisms with which such fungi must compete. To the mycologist and the student of soil fungi, certain chapters in the book are of more importance than those dealing strictly with diseases and their causal organisms.

Current methods of studying the fungi of the soil are severely criticized. The techniques of sampling macrofungi by quantitative studies of carpophore production are likened to studying "the vegetation above ground of a meadow solely by means of samples taken from a haystack." Such sampling obscures all variations of the mycobiota. The two problems usually stressed in the study of the microfungi of soils are (1) whether fungi occur as spores, resting cells, or active mycelium and

(2) the enumeration of these fungi. These problems are becoming more and more unreal as questions remain unanswered. Direct observation of soil fungi amounts to observation of actively growing mycelium rather than actively sporulating structures. "With the plate count method one identifies what one cannot see (i.e. *in situ*), whereas with the direct method one sees what one cannot identify." A more rational approach to the study of soil fungi is suggested in the form of a study of substrate relationships. A number of groups on this basis have been proposed, including saprobic sugar fungi, cellulose-decomposing fungi, lignin-decomposing fungi, predatory fungi, etc. The orderly development of a succession of such fungi, as a certain substrate is used, will become better understood as techniques for fungal detection improve. The demonstration of this succession by gross agar plate techniques will lead to confusion.

The *inoculum potential*, "the energy of growth of a fungus (or other microorganism) available for colonization of a substrate at the surface of the substrate to be colonized," required for infecting roots is greater than that for leaves, where a single spore can initiate infection.

In connection with the biological control of root disease fungi in the soil, the resistance of fungi like *Trichoderma viride* to various soil disinfectants is described. In discussing the significance of antibiotic production by soil microorganisms, it is pointed out that proponents of the idea that antibiotics produced by soil organisms become diluted in the soil have not taken into consideration the fact that the antibiotic is required only in the immediate vicinity of the very fine threads of mycelium producing it.

Pathogenic soil-inhabiting fungi have been defined as primitive parasites that can survive indefinitely as soil saprobes. Primitive parasites include those fungi capable of infecting immature or aging roots of seedlings or plants. Since successful parasitism involves a minimum disturbance to host tissues, the efficiency of a parasite can be correlated with the degree of symbiosis the parasite achieves with its host. Thus the most successful parasites are the ectotrophic mycorrhizal fungi.

The manner and significance of the *ectotrophic growth habit* is considered. The significance of this habit in root-infecting fungi "lies in its manifestation of an ability for continuous and indefinite spread over the host root system." This growth and spread are not checked by host resistance, although under certain circumstances they may be checked by *Trichoderma viride* if this fungus happens to be in the rhizospere biota. The energy and nutrients for the inoculum potential of root-infecting fungi with the ectotrophic growth habit, occur in resting

mycelium in infected host tissues, or in sclerotia in the soil. In the earlier experiments ending in failure, inadequate inocula were used; this led to the finding of the importance of a *food base* for root infection.

Garrett is of the opinion that "the very term 'obligate parasite' is not so much an objective biological definition, as a subjective admission of human failure to culture such organisms *in vitro*." He suggests that the root-inhabiting fungi demonstrate an intermediate type of behavior, and suggests the term *ecologically obligate parasites* for them. Such fungi grow well in pure culture, but in nature they cannot compete with more active saprobic fungi for dead organic matter.

Competitive saprophytic ability (or saprophyticity) is defined as: "the summation of physiological characteristics that make for success in competitive colonization of dead organic substrates." High growth rate and rapid germinability of spores, good enzyme-producing equipment, production of antibiotic toxins, and tolerance of antibiotics produced by other microorganisms are characteristics which help to produce this ability.

Mycelial momentum may be defined as "the product of effective mass of fungal inoculum and the velocity of fungal growth outwards from that inoculum." The possession of this characteristic will aid a fungus in continuing colonization of the roots of trees in an orchard or plantation; and it will assist the lignin-using mushroom, *Agaricus campestris*, in colonizing new areas by the use of simple rhizomorphs.

Topics considered at length include substrate groups, mycorrhizae, the rhizosphere, vascular wilt fungi, "the Cambridge method" of studying competitive saprophytic ability, saprobic survival of root-infection fungi in herbaceous or woody host tissues, decoy plants, host resistance as contrasted with disease escape, and various problems associated with the epidemiology of root diseases.

"The degree of unrealized root damage is appreciated only after the spectacular results often obtained when crops are grown with really healthy root systems, following efficient soil disinfection."—WM. BRIDGE COOKE.

THE GENUS ACHLYA: MORPHOLOGY AND TAXONOMY, by Terry W. Johnson, Jr. xv + 180 pp., 22 plates, University of Michigan Press, Ann Arbor. 1956. Price, $4.50.

A third of a century has elapsed since the publication of Coker's monograph of the Saprolegniaceae. During that interval much study

has been devoted to the water molds and it has become known that they are not restricted to water but are common inhabitants of soils. *Achlya*, the largest and most frequently isolated genus, is commonly studied in elementary classes as a representative of the group, not rarely, it is to be feared, under the name *Saprolegnia*. A modern treatment of this important genus will therefore be welcomed in all laboratories where fungi are studied seriously.

A brief, but highly informative introduction covers the historical background of the family and the genus, and discusses the occurrence, methods of collection and of isolation and culture, preservation of cultures and taxonomic criteria. The final section, on terminology, is illustrated by a plate giving 53 clear, diagrammatic drawings which clarify admirably the characters utilized in the taxonomic treatment, which occupies the major portion of the book.

Three subgenera are recognized: *Centroachlya*, with 6 recognized species; *Subcentrica*, with 6 recognized species, one of them subdivided into two varieties; and *Achlya*, with 22 recognized species and appended descriptions of two as yet unnamed taxa. The name of the first subgenus is adopted from Coker; the second is apparently new and is unfortunate in that it suggests a section rather than a subgenus (Art. 31). The descriptions are clear, standardized in form and as brief as is consistent with inclusion of all essentials, and are followed by critical notes and comparisons with related species. All are illustrated on plates 2–22, assembled in back of the book and with facing explanatory legends. Eight additional species and one variety are discussed as doubtful and there is a section of excluded taxa, so that reference may be found to all names in the literature. There is a bibliography of over 200 titles and a complete index.

The author's species concept, discussed on pp. 1 and 2, is broad and allows for the variation under different environmental circumstances which he believes is characteristic of these fungi. As a result, a number of species have been reduced to synonymy. This treatment could be applied with advantage to other groups and seems to represent a desirable trend at the present time.

The printing, format and binding are excellent and the book has been carefully edited. I have noted but one error and that, although repeated, is minor. The book should be a most useful reference work for many years to come.—G. W. MARTIN.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to those who have been members in good standing of the Mycological Society of America for over a year immediately preceding submission of manuscript. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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